Regulation of vascular tone and renal function by endothelin in health and renal disease

Jane Goddard

Submitted for the degree of PhD

University of Edinburgh

2004

Abstract

Since its discovery in 1988 the powerful vasoconstrictor endothelin-1 (ET-1), has been widely implicated in the pathophysiology of renal and cardiovascular disease. ET antagonists have favourable effects in experimental models of these conditions and orally acting ET antagonists appear very promising in clinical trials of pulmonary and systemic hypertension. However, there is a paucity of human data regarding the role of ET-1 and its receptors both on renal function and in renal disease. In this thesis, using selective ET receptor antagonists, I have therefore explored the systemic and renal haemodynamic and renal tubular effects of ET-1, in a series of acute clinical studies.

After confirming, in a dose ranging study, that ETA receptor blockade produces vasodilatation in healthy subjects, I have shown, in patients with chronic renal failure (CRF), that selective ETA but not combined ETA/B receptor antagonism produces substantial reductions in blood pressure that are associated with renal vasodilatation, a reduction in EFF and a reduction in proteinuria, suggesting a renoprotective action. These data show that ET-1 plays a major role in regulating blood pressure and renal vascular resistance in CRF, consistent with activation of the ET system in this condition. By contrast, selective ETB receptor antagonism alone produced substantial systemic and renal vasoconstriction in both healthy subjects and CRF. Thus the effects of ETB receptor blockade, whether in the presence of ETA receptor blockade or not, suggest the net effect of ETB receptor activation on the circulation, in health and renal disease, is to produce vasodilatation. Clearance of ET-1 from the circulation is also reduced during ETB receptor blockade and studies of urinary ET-1

excretion under conditions of ET receptor blockade suggest that renal tubular excretion of ET-1 is at least partly mediated through the ETB receptor.

I have also explored the interaction between the ET and renin-angiotensin systems, demonstrating that, while ETA receptor antagonism does not alter the pressor effect of angiotensin II, prior ACE inhibition will enhance the effect of ETA receptor blockade in healthy subjects to produce a picture similar to that seen in CRF, an action mediated by nitric oxide, and abolished by concomitant ETB receptor blockade.

In summary,these studies confirm a role for ET-1 in the pathophysiology of systemic and renal vasoconstriction in CRF and suggest that ETA, rather than combined ETA/B receptor antagonism is potentially the most useful stratagem for ET system blockade in the treatment of CRF, particularly in combination with ACE inhibitors. Chronic studies are now needed in patients with CRF to confirm this potential.

Declaration

I declare that all the work presented in this thesis is my own except where stated below, and it has been entirely composed by myself.

1. Studies

All clinical studies were performed by myself with the exception of Studies 1 & 2 (Chapters 3 & 4) which were performed as joint studies in conjunction with Dr JC Spratt and Dr IB Wilkinson.

2. Assays

Because of the very large number of samples produced by clearance studies requring analysis, these were performed in conjunction with the laboratory staff of the Clinical Pharmacology Unit*. All immediate processing of samples was undertaken by me. The subsequent analyses can be devided into those I undertook completely (flame photometer measurements, inulin measurements, haematocrit, osmolality), those I performed to gain experience of the assay but which were largely analysed by the laboratory staff (PAH, BQ-123, ET-1, ANG II) and those which I observed but were all analysed by the laboratory staff (PRA, Aldosterone, serum ACE activity, urinary protein). Cyclosporin was performed in the main hospital laboratory.

*Mr NR Johnston, Miss FE Howe, Miss L Coppard

Acknowledgements:

Special thanks must go to:

The Clinical Pharmacology Unit laboratory staff, Mr NR Johnston (senior MLSO), Miss FE Howe and Miss L Coppard for all their support in analysing the large quantities of samples these studies produced with cheerfulness, efficiency and good humour

Dr Andrew Rankin for his enthusiastic backing and Pfizer Global Research & Development, Sandwich UK for their financial support.

Professor Ton Rabelink for his critical appraisal of the renal studies and advice

Professor Allan Cumming for his ongoing encouragement and renal back-up in a clinical pharmacology setting

Finally, Professor David Webb, who afforded me the opportunity to undertake this research in the first place, who has been unstinting in his backing, advice and support.

Abbreviations

ACE	Angiotensin converting enzyme
ANG II	Angiotension II
ARAS	Atherosclerotic renal artery stenosis
AVP	Arginine vasopressin
CAD	Coronary artery disease
CHF	Congestive heart failure
CI	Cardiac index
CO	Cardiac output
COX	Cyclooxygenase
CRF	Chronic renal failure
CTF	C-terminal fragment
CyA	Cyclosporin
ECE	Endothelin converting enzyme
EFF	Effective filtration fraction
EPO	Erythropoietin
ERBF	Effective renal blood flow
ERPF	Effective renal plasma flow
ERVR	Effective renal vascular resistance
ET	Endothelin
FeNa	Fractional excretion of sodium
FWC	Free water clearance
GFR	Glomerular filtration rate
Hct	Haematocrit
HPLC	High performance liquid chromatography
IHD	Ischaemic heart disease
IVC	Inferior vena cava
IMCD	Inner medullary collecting duct
L-NMMA	L-N^G-monomethyl-arginine
LV	Left ventricle
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
NE	Noradrenaline
NO	Nitric oxide
NOS	Nitric oxide synthase
PAH	Para-aminohippurate
PHT	Pulmonary hypertension
PIH	Pregnancy induced hypertension
PET	Pre-eclampsia
PVD	Peripheral vascular disease
RBF	Renal blood flow
SAH	Sub-arachnoid haemorrhage
SVRI	Systemic vascular resistance index
UFR	Urinary flow rate
UNaV	Urinary sodium excretion

CONTENTS

Chapter 1

Introduction

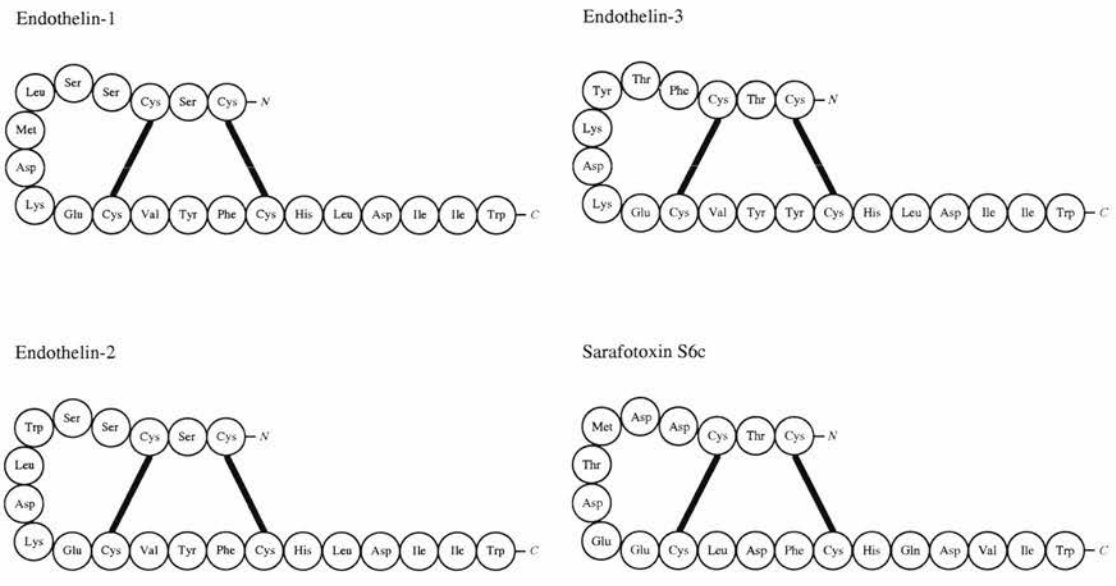
1.1 Introduction

Since its discovery in 1988, endothelin (ET) has been widely implicated in the pathophysiology of renal and cardiovascular disease. ET receptor antagonists have favourable effects in experimental models of these conditions and have proved useful in elucidating the role of the ET system. Orally acting ET receptor antagonists appear promising in clinical trials in pulmonary and systemic hypertension, but there is a paucity of human data regarding the role of ET and its receptors both on renal function and in renal disease. This thesis therefore explores the systemic and renal haemodynamic and renal tubular effects of ET, using selective ET receptor antagonists.

1.2 Biology of Endothelins

The endothelins are a family of 21 amino acid peptides with powerful vasoconstrictor and pressor properties that were first described by Yanagisawa in 1988 [1]. Three different isopeptides, ET-1, ET-2 and ET-3, have so far been identified, each with distinct genes and tissue distributions [1-3]. Each has a common structure of a hydrophobic C terminal end and two disulphide bonds within the peptide that form a loop [2, 3]. ET-2 differs from ET-1 by 2 amino acids and ET-3 from ET-1 by 6 amino acids. Endothelins are highly conserved peptides that show a similarity to sarafotoxins, cardiotoxic constrictor snake venom peptides produced by the Israeli burrowing asp *Actractaspis engaddensis* [4] (Fig 1.1).

Figure 1.1 Structure of endothelins and sarafotoxin S6b



Of the three peptides, ET-1 is the major endothelial isoform and is thus likely to be the most important in the regulation of vascular function. Its main site of production is the vascular endothelial cell but it is also produced by other cell types including renal tubular and mesangial cells, vascular smooth muscle cells and epicardial cells. (Table 1.1).

1.2.1 ET-1 biosynthesis

The gene product is the 212 amino acid pre-pro-ET-1. Regulation of the production of ET is thought to be at the level of gene transcription. Enhanced gene transcription occurs with a wide range of stimuli including other vasoactive hormones such as angiotensin and vasopressin [20-22] cytokines such as interleukin-1, platelet derived growth factor, transforming growth factor beta [23, 24], endotoxin [25, 26] hypoxia [27], insulin [28] oxidized low density lipoprotein [29] and cyclosporin (CyA) [30]. In contrast, prostacyclin [31], nitric oxide [32], the natriuretic peptides [33], oestragen [34] and heparin all inhibit gene transcription.

Table 1.1 sites of ET biosynthesis

ET-1	ET-2	ET-3
Vasculature - endothelial cells [5]; - vascular smooth muscle cells [6]	Vasculature - endothelial cells [5]	Gut [7, 8] Adrenal secretory tissue [14]
Heart (epicardium) [9]	Heart [10]	
Kidney [12, 13]	Kidney [11]	Kidney [11]
Lung (bronchus) [15]		
Central and peripheral nervous system [17, 18]		Central nervous system [19]
Macrophages [16]		Macrophages [16]

Pre-pro-ET-1 is cleaved by intracellular furin-like proteases to proET-1 then big ET-1 (38 amino acids) which is largely, but not wholly, biologically inactive [35]. Endothelin converting enzyme (ECE), a metalloproteinase [36], then splits big ET-1 to the biologically active ET-1 and C-terminal fragment (CTF). Two ECEs have been identified, ECE-1, existing in four distinct isoforms [37-39] and ECE-2 [40], both of which preferentially catalyse the cleavage of big ET-1. Each ECE has a different tissue distribution and different pH at which it acts [37, 41]. ET-1 can also be produced from big ET-1 by chymases [42], which can also cleave big ET-1 to produce the vasoconstrictor ET-$1_{1\text{-}31}$ [43], and non-ECE metalloproteases. ET-1 secretion from endothelial cells is largely abluminal, towards the adjacent vascular smooth muscle [44] (Fig 1.2).

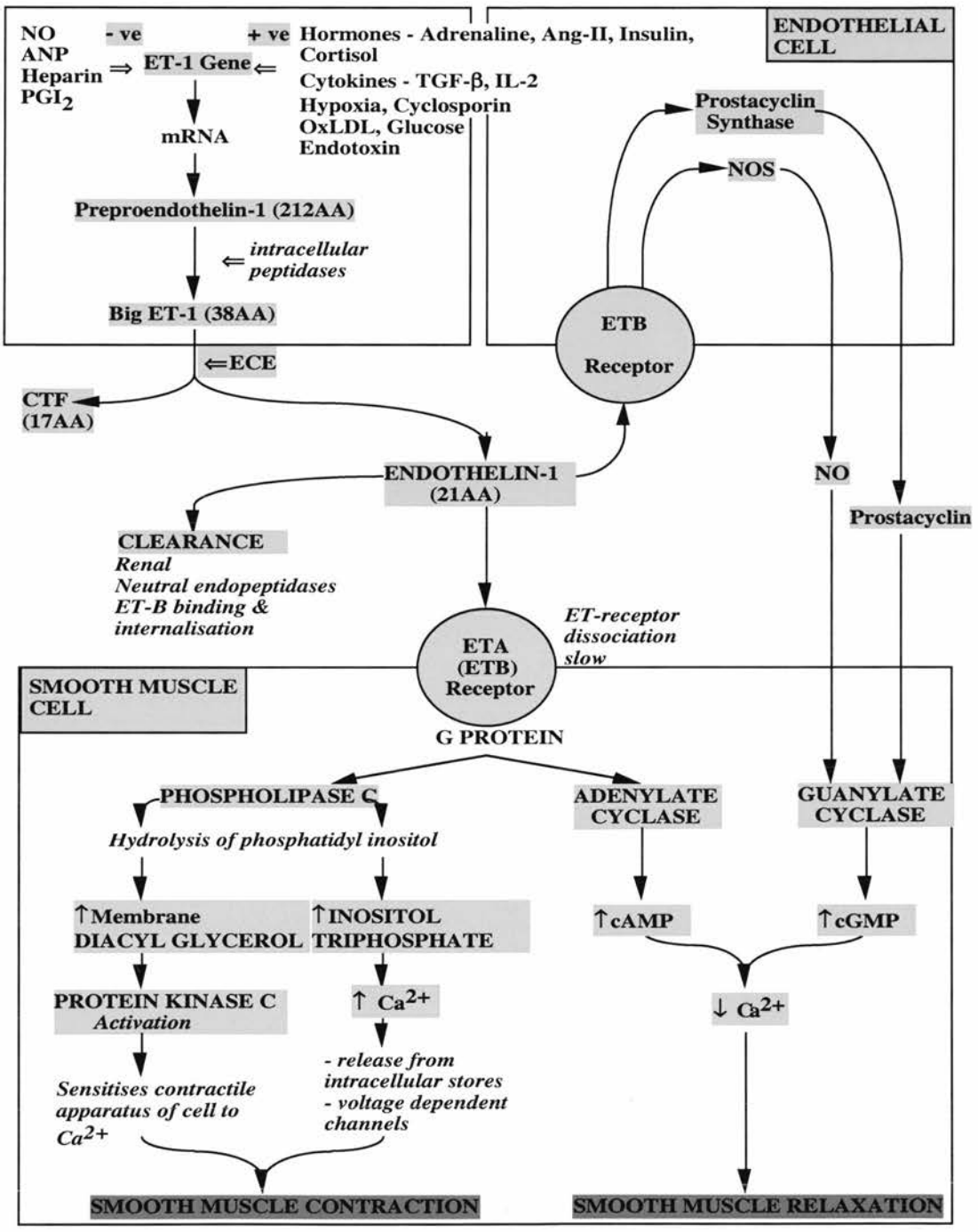
1.2.2 ET receptors

Two ET receptors have been identified and cloned in mammals. Both are seven domain trans-membrane proteins. ETA receptors [45] have a high affinity for ET-1 compared to ET-2 and ET-3, whereas ETB receptors [46] have an equal affinity for all three ET peptides. Within the circulation, ETA receptors are found on vascular smooth muscle cells [45, 47] and their activation results in vasoconstriction. ETB receptors are also found on vascular smooth muscle cells [48], where they can mediate vasoconstriction, but are primarily found on the vascular endothelium [49] where their activation results in vasodilatation via the nitric oxide (NO) system [50] and prostacyclin [51] (Fig 1.2). Within the kidney, in man, ETA receptors are localised to vascular smooth muscle, notably in the glomeruli, vasa recta and arcuate arteries. ETB receptors are more numerous (ETB to ETA ratio 2:1) and more widespread with a high concentration in the collecting system [11, 52].

1.2.3 ET receptor signalling

Signal transduction is via coupling to a G protein [53] and activation of intracellular phospholipase-C with subsequent hydrolysis of phosphatidyl inositol [54, 55], activation of protein kinase C [56] and changes in intracellular calcium [57, 58]. Binding of ET-1 to its receptors is irreversible, largely due to the internalisation of the receptor-ligand complex, and produces slow onset, sustained actions (Fig 1.2).

Figure 1.2 ET-1 biosynthesis



1.2.4 Actions of ET-1

Endothelin was originally identified as a vascular endothelial cell derived peptide acting on vascular smooth muscle cells to produce a biphasic response on blood vessels of a transient ETB receptor mediated vasodilatation [59] followed by a sustained ETA receptor mediated vasoconstrictor and pressor response [60]. However, in addition to its vasoconstrictor properties, it is clear now that it has many other functions in vascular and other tissues.

ET-1 can produce contraction of other cell types eg. airway smooth muscle cells producing bronchoconstriction [61], detrusor smooth muscle cells causing bladder contraction [62] and mesangial cells reducing the filtration coefficient [63]. ET-1 also functions as a powerful mitogen promoting cell growth in a wide variety of cell types [63-65] and interacting with other growth factors (eg. vascular endothelial growth factor in vascular smooth muscle and endothelial cells [66]). It is pro-inflammatory [67, 68] modulating cytokine production (eg. TNF-α and interleukin-6 in mast cells [69], interleukin-1β in astrocytes [70]) and pro-fibrotic [71]. It modulates other hormone systems, (eg. atrial natriuretic peptide [72], angiotensin [73], noradrenaline (NE) [74], arginine vasopressin (AVP) [75], and aldosterone [76]) and also influences ion and fluid transport in the gut [77], lungs [78] and kidneys [79-81]. ET-1 can also act as a neuropeptide in the brain [82] and mediate pain nociception [83].

Knockout models have also demonstrated that ET is vital in normal embryonic development of tissues derived from the neural crest. ET-1 knockout mice die shortly after birth from craniopharyngeal malformations [84]. ETB receptor knockout mice have aganglionic megacolon and an abnormal coat colour demonstrating abnormal development of myenteric ganglion neurones and epidermal melanocytes [85].

1.2.5 ET clearance

The half-life of exogenously administered ET-1 in the circulation is ~1 min [25, 86]. Clearance of ET-1 from the circulation is by receptor and non-receptor mediated mechanisms. ETB receptor binding and internalisation with intracellular degradation accounts for the majority of clearance particularly in the pulmonary circulation, with splanchnic and renal circulations also contributing [86]. In rats ~70% of ET-1 is removed in the lungs, in man this is ~50% [87, 88]. Renal clearance [86] and enzymatic degradation by neutral endopeptidases on endothelial cell membranes [89] also occurs.

1.3 Plasma ET-1 concentrations

In studying the activity of the ET system, a marker of that activity should be sought. Throughout this thesis, plasma ET-1 concentration is given. However, in interpretation of its significance, it must be borne in mind that, though big ET and its cleavage products can all be detected in the circulation, most ET-1 is released from endothelial cells abluminally [44] where it acts on smooth muscle cells. Because of ET-1's paracrine action, plasma ET-1 concentrations may not be an appropriate measure of endothelial synthesis.

Also, plasma ET level will represent a balance between production and clearance. ET-1 is rapidly cleared from the circulation. Though it will exert effects for up to 60 minutes, the plasma half-life in normal subjects is about 1 minute [25]. Reductions in ETB receptor numbers or in renal function may reduce this clearance increasing plasma ET-1concentrations without altering production, though there is no evidence yet as to whether these elevated concentrations consequent upon reduced clearance are biologically active as opposed to simply immuno-competent and thus detected by current assays. Measurement of big-ET and CTF have been promoted as more accurate reflectors of endothelial synthesis because their removal from the circulation is slower and more consistent [90, 91]. ETB inhibition (i.e. reduced receptor clearance) results in a rise in plasma ET-1 but not big ET-1 or CTF i.e. no increase in production.

Finally, in considering the kidney, because less than 1% of injected radiolabelled ET-1 is recovered in the urine [92], neither glomerular filtration nor tubular secretion of plasma ET-1 accounts for urinary ET-1, which is therefore assumed to be primarily of renal origin. Thus urinary ET-1 excretion rate may be used as a marker of renal ET-1 production.

1.4 ET receptor antagonists

ET receptor agonists and antagonists have been used to define the role of the endogenous ET system and confirm its importance in cardiovascular function and dysfunction. Attention is now focusing increasingly on drugs that may have a role in clinical medicine. Though ECE inhibitors and monoclonal antibodies have been used experimentally, and the former may be a potential future therapeutic option, most clinical work to date has been with ET receptor antagonists.

ET receptor antagonists can be divided, according to their receptor selectivity, as ETA or ETB selective, and non-selective ETA/B receptor antagonists. The term ETA selective is generally used for compounds with >1000 fold selectivity for the ETA over the ETB receptor. It is important to appreciate that so-called 'non-selective' antagonists are still generally selective for the ETA receptor but the ratio of ETA to ETB effect is less than for the selective agents (Table 1.2). In practical terms, more work with these selective vs. non-selective receptor antagonists is needed to delineate the respective roles of ETA and ETB receptors in normal physiology and, more critically, in pathophysiology before conclusions can be drawn regarding which are likely to be most beneficial in a given clinical situation.

A further division, in terms of their clinical potential, must be made into orally active compounds and those available only as parenteral preparations. There will be certain situations where an intravenous preparation is preferred, notably to counter an acute, isolated insult (such as the cerebral ischaemia following subarachnoid haemorrhage or stroke, unstable angina, acute myocardial infarction, acute renal failure and post-operative pulmonary hypertension). However, many of the anticipated indications for the ET receptor antagonists currently in development (including hypertension,

chronic heart failure and coronary restenosis) will require chronic treatment and, thus, oral agents are more suitable (Table 1.2).

A series of peptide antagonists have been developed from the modification of the ET peptides to produce compounds that bind to, but do not activate the receptors. Cyclic peptides mimic the hairpin loop of the endothelins; BQ-123 is a cyclic pentapeptide, ETA selective receptor antagonist developed from BE 18257B (a natural product of *Streptomyces misakiensis*) [93, 94] and TAK-044, a cyclic hexapeptide, non-selective ETA/B receptor antagonist [95]. Modification to produce a series of linear peptides can generate ETB selective receptor antagonists such as BQ-788 [96]. As peptides, these agents are broken down in the gut and are thus only active parenterally, and then with only relatively short half-lives. Experimentally, the half-life of BQ-123 can be increased by side-chain modifications that reduce hepatic extraction [97], but the clinical applicability of this group of compounds remains limited by the lack of oral analogues.

Screening libraries for compounds with ET blocking activity, and molecular modelling, has resulted in a series of non-peptide antagonists with greater bioavailability. Ro 47-0203, bosentan [98], is a sulphonamide compound with non-selective receptor blocking activity that has the benefit of being available as both an oral and intravenous preparation. Practically, this would allow treatment to be started in the acute situation, say, an exacerbation of undiagnosed heart failure, with the intravenous preparation and continuing treatment thereafter with the oral formulation. SB 209670, a carboxylic acid derivative, is a more potent non-selective receptor antagonist but is only available as an intravenous preparation [99]. SB 217242 (enrasentan) is an analogue of this compound with improved bioavailability, currently in clinical studies as an oral drug [100]. Orally active and selective ETA receptor antagonists have also been developed. ABT 627 is a carboxylic acid derivative with 2000-fold selectivity for the ETA vs. the ETB receptor and a half-life of 24 hours in humans [101] making it theoretically a very attractive choice where chronic selective receptor antagonism is required. Minor modifications to this structure produce compounds highly selective for the ETB receptor [101]. TBC 11251 and TA 0201 are

sulphonamide derivatives with high bioavailabilities which are also highly selective for the ETA receptor [102]. TA 0201 has a half-life of only 0.9 hours in pharmacokinetic studies but generates an active metabolite with demonstrable inhibitory effect at 8 hours [103].

Table 1.2: Endothelin receptor antagonists in development

1.2a: ETA selective endothelin receptor antagonists

Name	Route	ETA selectivity*	Stage of Development	Anticipated indication	Manufacturer
TBC 11251 (Sitaxentan)	O + IV	x7000	Phase III Phase II	PHT CHF	Encysive/ Texas Biotechnology
LU 135252 (Darusentan)	O +IV	x130	Phase III Preclinical Unknown	CHF Arrhythmias HT	Knoll
ABT 627	O	x2000	Phase I	Coronary restenosis	Abbott
TA 0201	O	x2700	Phase I Preclinical	CHF PHT, BPH	Tanabe Seiyaku
ZD 1611	O	x1000	Phase I Preclinical	PHT CHF, HT, COPD	AstraZeneca
RO 611790	IV	x1000	Phase I	Renal failure, solid tumours	Roche
S 0139	IV	NK	Phase II	Cerebral vasospasm, neuroprotection	Shionogi
BQ-123	IV	x2500	Preclinical	Arrhythmias, IHD, cerebral ischaemia, HT, PVD, CHF	Banyu
PD 147953	IV	x7000	Preclinical	Coronary disease	Fujisawa (Parke-Davis)

1.2b: Non-selective endothelin receptor antagonists

Name	Route	ETA selectivity*	Stage of Development	Anticipated indication	Manufacturer
RO 470203 (Bosentan)	O + IV	x20	US licence Phase III Phase II Preclinical Unknown	PHT CHF HT DM neuropathies, Ischaemia/ reperfusion injury IHD	Roche (Actelion)
SB 217242 (Enrasentan)	O	x110	Phase II Phase I Preclinical	PHT CHF Restenosis, CRF, stroke	SmithKline Beecham
J 104132	O	x5	Phase I Preclinical	HT CHF	Merck & Co
TAK-044	IV	x18	Phase II Phase I Preclinical	IHD, CRF, SAH HT CHF	Takeda (Parke-Davis)
RO 610612 (Tezosentan)	IV	NK	Phase II/III Preclinical	CHF, HT Reperfusion injury	Roche (Actelion)
PD 145065	IV	x4	Preclinical Unknown	HT, CRF Coronary disease	Parke-Davis

Key: O - Oral, IV - intravenous, NK - not known, * based on binding and functional assays BPH - benign prostatic hypertrophy, CHF - Congestive heart failure, COPD - Chronic obstructive pulmonary disease, CRF - chronic renal failure, DM - diabetes, HT - hypertension, IHD - ischaemic heart disease, PHT - pulmonary hypertension, PVD - peripheral vascular disease, SAH - sub-arachnoid haemorrhage

A potentially interesting preclinical development is the description of L-747072, an *angiotensin* receptor antagonist with non-selective ET receptor antagonist activity, providing a possible dual target approach for the treatment of hypertension and other cardiovascular conditions [104].

1.5 Endothelin and the kidney

1.5.1 Physiology

Within the kidney, ET-1 is synthesised by endothelial, mesangial, glomerular epithelial and medullary collecting duct cells [13, 105-107] and may act as a paracrine/autocrine regulator of renal and intrarenal blood flow, glomerular haemodynamics and sodium and water homeostasis [80, 108]. Though the tubular effects are probably intra-renal and paracrine in nature, and ET-1 probably also acts locally to regulate glomerular haemodynamics, there is some evidence that circulating ET may also be important in renal functional changes in cardiorenal states of ET activation such as heart failure [109].

In animals, exogenous ET-1 causes renal vasoconstriction, sometimes preceded by transient vasodilatation [110-113]. Indeed, the renal vasculature is more sensitive to the vasoconstricting effects of ET-1 than other vascular beds [114]. Studies suggest that this vasoconstriction is primarily cortical [115, 116] with an initial medullary vasodilatation [115, 117]. In man, the effect of exogenous ET-1 on the renal vasculature is to cause vasoconstriction, a fall in glomerular filtration rate (GFR), and an increase in filtration fraction [118-120].

There are abundant ET receptors in the kidney but the roles of the ET-A and ET-B receptors may show a species difference. In pigs, rabbits and dogs, ET-A inhibition blocks the vasoconstricting effects of exogenous ET-1 [59, 121-123], while, in dogs, administration of an ET-B agonist causes vasodilatation [124] and ET-A inhibition unmasks a presumably ET-B mediated vasodilatation in response to ET-1 [123]. Conversely, in rats there is some evidence to support ETB as well as ETA receptor mediated renal vasoconstriction. ET-B agonist administration has been shown to causes renal vasoconstriction [125] and combined ETA/B inhibition to abolish whereas ETA receptor blockade only attenuate ET-1 induced vasoconstriction [126, 127].

Studies of intrarenal changes in blood flow suggest that the observed cortical vasoconstriction is mediated by ETA receptors [115, 116] whereas the medullary vasodilatation is ETB receptor mediated and NO dependent [115, 117].

In respect of tubular functions, the highest concentrations of ET-1 are found in the medulla. [128, 129], and there is now a substantial body of evidence supporting a role for ET-1 in the renal regulation of volume homeostasis. ET-1 is produced by inner medullary collecting duct cells (IMCD) where it inhibits the AVP stimulated retention of water [80], and extra-cellular sodium concentrations may regulate IMCD ET-1 production [108]. Additionally, the ETB receptor appears to have a natriuretic role at least in animals. ET-1, acting via ETB and NO can inhibit chloride transport, thus promoting natriuresis, in the medullary thick ascending limb of Henlé [130, 131]. Additionally, *in vitro* low dose picomolar concentrations ET-1, binding to ETB receptors, will activate amiloride-sensitive sodium channels in distal tubular cells, though higher, nanomolar doses inhibit this channel by a non-ETB receptor dependent mechanism [132]. *In vivo*, dogs subjected to high-grade ETA blockade demonstrate a vasodilatation and natriuresis in response to administration of exogenous low dose ET-1presumed to be an unmasking of an ETB receptor effect [123] and ETB receptor blockade shifts the pressure diuresis and natriuresis response to the right in rats (ie. a greater renal perfusion pressure is needed to excrete the same salt and water) [133]. Finally, ETB knockout mice, and ETB antagonist treated rats, develop a sodium dependent hypertension [134, 135], and collecting duct-specific ET-1 knockout mice develop salt retention and hypertension on a high salt diet [136].

In man, the effect of exogenous ET-1 on the renal vasculature is to cause vasoconstriction, a fall in glomerular filtration rate (GFR), an increase in filtration fraction and a profound retention of salt and water, probably as a consequence of the renovascular changes rather than any direct ET-1 effect. [118-120]. Within the human kidney, ET-A receptors are localised to vascular smooth muscle, notably in the glomeruli and vasa recta and arcuate arteries. ET-B receptors are more numerous (ET-B to ET-A ratio 2:1) and more widespread with a high concentration in the collecting

system [11, 52]. The distribution of the receptors in man would suggest a vasoactive role for ET-A receptors and a role in sodium and water handling for ET-B receptors.

Human studies comparing exogenous ET-1 and ET-3, as ET-A and ET-B receptor agonists respectively, suggests that ET induced renal vasoconstriction is ET-A receptor mediated [137]. However, renal vasoconstriction with ET-3 has also been demonstrated, although at high dose, suggesting a possible constrictor role for ET-B receptors [138]. ET-A blockade can counter the effects of exogenously administered ET-1 on renal blood flow (RBF) and GFR but has no effects alone on renal function [120, 139-141] suggesting again that ETA receptors mediate ET-1 induced renal vasoconstriction, but that endogenous ET-1 has little role in the maintenance of normal renovascular tone. However, administration of the non-selective ETA/B receptor antagonist SB209670 does produce a significant increase in RBF compared to placebo [142]. No studies in man to date have demonstrated significant effects on salt and water homeostasis.

Thus, the paucity of work with antagonists in the human kidney has left significant uncertainties regarding the importance of endogenous ET and the roles of the receptor subtypes in the kidney in human physiology.

1.5.2 Pathophysiology

ET-1 has been widely implicated in renal disease, both in acute renal failure eg. ischaemia induced renal injury, CyA nephrotoxicity and radiocontrast nephrotoxicity [143-147] and progressive renal dysfunction [148].

Radiocontrast nephrotoxicity

As contrast induced nephrotoxicity is characterised by intense renal vasoconstriction, ET-1 has been proposed as a potential mediator of this condition. Contrast media increase ET-1 gene transcription in endothelial cells [149] and increases in both plasma and urinary ET-1 have been observed after contrast media administration in animals [149, 150] and in patients with CRF [151]. Additionally, non-selective ET receptor antagonism abolishes contrast induced renal vasoconstriction in dogs [152],

as does selective ETA receptor antagonism in rats [153]. Selective ETA receptor antagonism also prevents contrast induced renal medullary hypoxia in rats [154]. However, in patients with CRF undergoing cardiac angiography, ET receptor antagonism with SB 209670 was associated with a worse outcome than hydration alone [155].

Ischaemic acute renal failure

Hypoxia can increase ET-1 production in renal tubules [156]. Increased ET-1 peptide expression is seen in the renal vasculature and in the cortical tubular epithelium of kidneys after ischaemia reperfusion [157]. Specifically, ET-1 increases in renal medullary interstitial cells, damaged tubules at the corticomedullary junction and peritubular capillaries surrounding these damaged tubules. An increase in renal cortical and medullary ETA and cortical ETB receptors can also be demonstrated within 24hrs of ischaemia, with changes being maximal at 8 days. By contrast, medullary ETB receptors decrease [158].

ET receptor antagonists have ameliorated ischaemia-reperfusion injury in animal models [147, 157, 159-162] and can improve the warm [163] and cold [164] ischaemic injury that occurs during transplant retrieval. As selective ETB antagonists are not protective in ischaemia-reperfusion [165], it appears that ET-1 mediated vasoconstriction via the ETA receptor is the pathogenic mechanism abrogated. There is also some evidence from knockout models that the ETB receptor is involved in the renal recovery after ischaemia reperfusion [166], an observation supported by a study demonstrating that non-selective ETA/B, but not selective ETA receptor blockade during ischaemia reperfusion is associated with increased tubulointerstitial fibrosis and proliferation at 6 months [167].

Cyclosporin nephrotoxicity

CyA is associated with both acute and chronic renal dysfunction, the former probably consequent upon changes in renal haemodynamics, the latter associated with irreversible structural changes. CyA is well documented as causing acute renal vasoconstriction in animal models with a reduction in RBF and GFR. [168]. *In vitro*,

CyA has been shown to increase gene transcription and secretion of ET-1 from endothelial and smooth muscle cells [169-171]. CyA treatment increases ET-1 in proximal tubules in rats [172] and CyA causes down regulation of ET receptors in the kidney [173]. Increased activity of the ET system is also indicated by the ability of ET receptor antagonism to attenuate CyA induced smooth muscle cell contraction [171] and CyA induced renal vasoconstriction [145].

In vitro, ET receptor antagonism attenuates CyA induced smooth muscle cell contraction [171]. *In vivo*, ET receptor antagonists can improve acute CyA induced renal vasoconstriction in animal models [144, 145, 174-176], and a similar attenuation of CyA induced fall in RBF, but not blood pressure rise, has been demonstrated in healthy men with bosentan [177]. Plasma ET-1 concentrations [178] and big ET-1 concentrations [179] are elevated in patients on CyA, but, as yet, no studies with ET receptor antagonists have been performed in patients on chronic treatment.

Chronic renal failure

Beside its haemodynamic effects ET-1 has mitogenic and pro-fibrotic properties that may play a role in CRF. In support of a role for ET-1 in CRF, low renal mass models are associated with increased production intra-renally, both in vascular and renal tissues, of ET-1 with a positive correlation between ET-1 levels and glomerulosclerosis [148, 180-182] apparent indirectly in increased urinary ET-1 excretion [180, 182]. Also combined ETA and ETB blockade, drops the filtration fraction whilst maintaining the GFR [134] raising the possibility that ET promotes hyperfiltration with its consequent potential for renal injury.

ET receptor antagonists have been shown to prevent or attenuate the progression of renal insufficiency in a rat remnant kidney model [182-185]. While non-selective ETA/B receptor antagonists have produced positive results [183], the effect is less than that seen with selective ETA receptor antagonism [185], and concomitant administration of a selective ETB receptor antagonist abolishes the beneficial effects of ETA receptor antagonism [184]. A reduction in cortical ETB mRNA receptor

expression by contrast with an increase in ETA receptor and ET-1 expression may explain this differential response [184], and have implications for the use of non-selective ET receptor antagonists in CRF.

Human data supporting a role for ET-1 in CRF progression is more circumstantial. In patients with autosomal dominant polycystic kidney disease, intra-renal ETA mRNA is increased 5-10-fold compared to healthy controls [186]. Plasma and urinary ET-1 concentrations in patients with nephrotic syndrome due to focal segmental glomerulosclerosis are significantly higher than in normal controls [187] and nephrotic patients who show a reduction in proteinuria with immunosuppressive therapy also show reductions in urinary ET-1 excretion [188]. Urinary excretion of ET-1 is doubled in patients with CRF compared to healthy controls. This occurs alongside a reduction in prostaglandin I_2 urinary metabolites suggesting that a vasoconstrictor-dilator balance present in healthy kidneys is disrupted in CRF [189].

While the above points to the therapeutic potential of ET receptor antagonists in CRF, to date only one interventional study has been performed in patients. Type I diabetics with proteinuria but essentially normal renal function treated for 6 weeks with an ETA receptor antagonist showed no alterations in RBF but did have a reduction in proteinuria, a potentially renoprotective effect [190].

1.6 Endothelin and the cardiovascular system

1.6.1 Physiology

The importance of ET-1 in cardiovascular function is suggested by the actions of exogenous ET-1. In respect of the vasculature, *in vitro*, activation of vascular smooth muscle ETA receptors causes smooth muscle contraction and blood vessel constriction, and the administration of exogenous ET-1 is associated with a sustained increase in blood pressure and peripheral vascular resistance [59, 60, 119]. In animals, this is preceded by a transient period of hypotension and vasodilatation [59, 60] mediated by ETB receptors [59].

In man, forearm models in healthy volunteers have shown that ETA receptor antagonism causes local vasodilatation [191-195] consistent an important role for ET-1 in the maintenance of normal vascular tone via the ETA receptor. These forearm findings are disputed [196, 197], but differences may be attributable to concentrations of antagonists used [198]. However, acute systemic studies in healthy men [139, 199, 200] have failed to demonstrate a significant effect of ETA receptor antagonism on basal haemodynamics. Similarly, while non-selective ETA/B receptor antagonism with TAK-044 has been shown to cause vasodilatation and a reduction in blood pressure in healthy volunteers [201], studies using the non-selective antagonists bosentan [202], and SB 209670 [142] have demonstrated little effect on systemic haemodynamics in healthy subjects. Thus, while the ETA receptor mediates ET-1 induced vasoconstriction, the role of the ETA receptor in the maintenance of vascular tone is not proven.

ETB receptors can be both vasodilator (endothelial) and vasoconstrictor (vascular smooth muscle) in function. The balance of these two functions in health has yet to be fully elucidated. Selective ETB receptor agonists can cause constriction *in vitro* and increase blood pressure *in vivo* [203-205], but the relative importance of vasoconstrictor ETB receptors appears to vary between species and vessel type [206].

In man, ETB agonists cause constriction of forearm resistance and hand capacitance vessels in vivo in healthy subjects [207, 208], suggesting that ETB receptors also mediate ET-1 induced vasoconstriction. However, the vasoconstriction produced by exogenous ET-1 can be abolished by ETA receptor blockade alone both locally [209], and systemically [139]. Forearm studies have also demonstrated vasoconstriction in response to ETB receptor blockade in healthy volunteers [194, 196] and an attenuation of the vasodilator actions of ETA receptor blockade when ETB receptor antagonists are concurrently administered [194]. Similarly, systemic administration of ETB receptor antagonists produces increases in systemic vascular resistance [210] consistent with the net result of ETB receptor activation being vasodilatation in normal physiology.

The direct effects of ET-1 on the heart are probably species specific but a positive inotropic response to ET-1 is seen in isolated cardiac tissue from rats and humans [211, 212], and in *in vivo* models where coronary vasoconstriction is countered with a vasodilator [213]. The integrated haemodynamic response is, however, much more complicated. ET-1 associated increased peripheral vascular resistance and blood pressure produce reflex reductions in HR and cardiac output and coronary vasoconstriction can cause myocardial ischaemia and cardiodepression.

1.6.2 Pathophysiology

1.6.2a Hypertension

With respect to pathophysiology, in addition to its haemodynamic actions, exogenous ET-1 has been demonstrated *in vitro* to be mitogenic, thus causing vascular remodelling and cell proliferation, and causes renal sodium retention [118], features of hypertension. Production of ET-1 is increased in some (such as the Dahl salt-sensitive and the stroke-prone spontaneously hypertensive rat), but not all rat models of hypertension. Those models where ET-1 production is increased (mostly, but not exclusively salt-dependent types) are associated with increased vascular growth and a response to both selective and non-selective ET receptor antagonists. This response comprises not only a modest reduction in blood pressure but also a marked regression of vascular growth [214]. Altered intrarenal ET-1 production may contribute to essential hypertension. Animal studies indicate that tubular ET-1 has an important role in the physiological regulation of sodium excretion and volume status and hence blood pressure. Knockout mice with no collecting duct ET-1 retained sodium and developed salt sensitive hypertension [136] and ET-1 produced by IMCD also inhibits the AVP stimulated retention of water [80]. Urinary ET-1 excretion is reduced in hypertensives compared to normal controls suggesting that either renal ET-1 synthesis is reduced or breakdown is enhanced [215, 216]. Hence ET nephron production or handling may be deranged in essential hypertension, there may be inappropriate sodium and water retention aiding the development or maintenance of hypertension. Spontaneously hypertensive rats provide an

experimental model of this in that they have reduced medullary ET levels after the development of hypertension [217].

In man, elevated plasma ET concentrations are found in hypertension, though this is not consistent [218]. These high concentrations would appear, mostly, to be a feature of severe hypertension or indicative of the presence of complications or co-existing disease. Prepro ET-1 and ECE mRNA is increased in the vascular smooth muscle cells of hypertensive patients [219], and there is also evidence to suggest that endothelial function, specifically endothelium-dependent vascular relaxation, is impaired both in hypertension, and in groups at risk of hypertension [220, 221].

Forearm studies with ETA and ETB receptor antagonism suggest increased vascular ET activity in patients with essential hypertension compared to normotensive controls, (though this has not been confirmed by other groups [222]) and a greater response to non-selective antagonism compared to selective ETA antagonism [196, 223], suggestive of a possible increase in the importance of vascular smooth muscle vasoconstrictor ETB receptors in disease.

To date, one major study has examined the anti-hypertensive effects of ET receptor antagonism in man. In 293 hypertensive patients, bosentan at a dose of 500 to 2000 mg/day for 4 weeks produced a reduction in blood pressure equivalent to that with 20 mg of enalapril [224]. This reduction was achieved without a reflex increase in activity of the sympathetic nervous system (as measured by plasma NE levels and HR) or renin-angiotensin system. Further confirmatory work is needed but, given the number of different anti-hypertensive drugs already available, a new class of drug would have to demonstrate greater efficacy, a better side-effect profile, or actions additional to its blood pressure lowering abilities. It is here that potential benefits in terms of reduction in long-term structural changes in the blood vessel (vascular hypertrophy or atherogenesis) or myocardium, an amelioration in endothelial dysfunction, or a beneficial effect on salt and water balance, might accrue. Hypertensive sub-groups such as salt sensitive patients, or black hypertensives (with

higher plasma ET levels and impaired endothelium-dependent relaxation [221, 223, 225]) might potentially be targeted.

Chronic renal failure and hypertension

Hypertension in CRF is a major cause of morbidity and mortality. ET-1 has been implicated as a mediator of this hypertension by a number of mechanisms. Firstly, as a consequence of reduced renal clearance, plasma ET concentrations are certainly consistently elevated in CRF [216, 226-228]. The effect of exogenous ET-1 on the renal vasculature is to cause vasoconstriction (which will activate the renin-angiotensin system) and profound salt and water retention, both having the potential to raise blood pressure [118]. It is not proven yet whether the elevated concentrations of ET-1 seen in CRF represent biologically or just immunologically competent peptides but in an animal model where exogenous ET-1 was administered to bilaterally nephrectomised rats, there was a prolonged rise in blood pressure compared to sham-operated rats associated with an increased plasma half-life of ET-1 [229] suggesting that there is potential for elevated plasma ET concentrations to cause hypertension.

Secondly, renal ET-1 production [181] and urinary excretion [216] are increased in CRF. Additionally, in experimental nephritis associated with mesangial proliferation, the renal vasculature is more sensitive to the constricting effects of ET than in normal kidneys [230] hence an amplification of the renovascular effects of ET-1 could be envisaged in CRF, an effect that could be exacerbated by the higher plasma ET concentrations due to reduced renal clearance. ETB receptors appear to be important in protecting against hypertension in CRF. In a 5/6 nephrectomy remnant kidney model of CRF, ETB receptor deficient rats have higher renal ET-1 content, a higher blood pressure and faster progression in renal failure than wild type animals [231].

Erythropoietin induced hypertension

Recombinant human erythropoietin (EPO) is widely used to improve the haematocrit of patients with CRF and is associated with a rise in blood pressure [232, 233]. *In vitro*, EPO has been shown to increase production of ET-1 from endothelial cells [234]. In transgenic mice overproducing EPO, increasing haematocrit to 80% is associated with increased ET-1 mRNA levels in aorta, liver, heart, and kidney, despite systemic vasodilatation [235]. In man, intra-arterial infusion of ET-1 causes less forearm vasoconstriction after EPO therapy than before [233]. If plasma ET were to be increased by EPO therapy, this might cause a down-regulation in receptors and thus a reduced response to exogenous ET. Thus a link between EPO associated hypertension and plasma ET has been sought.

Patient studies, however, are again conflicting. Some do not show an elevation in plasma ET after EPO treatment [233, 236, 237] but in two studies, chronic administration was associated with an eventual rise in plasma ET concentration, correlating additionally with blood pressure [232, 238].

Atherosclerotic renal artery stenosis (ARAS)

Lerman *et.al.* [239] surveyed patients with symptomatic atherosclerosis and found a significant correlation with plasma ET levels and the number of sites affected by atherosclerosis. More recently, ET-1 mRNA and ET-1 have been demonstrated not only in endothelial cells but also in cell rich areas of atherosclerotic plaques particularly foamy macrophages [240, 241]. Mechanical injury to vascular endothelium with a balloon angioplasty catheter has been shown to cause accumulation of ET within the injured vessel in animal studies and occlusion during angioplasty procedures released ET distally in human coronary arteries in vivo [241, 242]. Similarly, after aortic cross-clamping for atheromatous aortic aneurysm repair, increased levels of plasma ET have been observed [243].

In ARAS, atherosclerosis and ischaemia co-exist; the supplying vessel to the kidney will be atheromatous and the kidney ischaemic because of the stenosis. Thus, the possibility of a role for ET in the secondary hypertension of ARAS arises, a theory

supported by the observation that, in a rat model, ET antagonism will attenuate the rise in blood pressure that occurs after single renal artery clipping [244].

The elevation of plasma ET does not need to be systemic; an elevation in the post-stenotic renal vessels might suffice to have a sodium retaining effect via renal vasoconstriction that will generate hypertension, but one might expect to see changes in renal vein ET levels. Unfortunately, while some studies in man have shown at least a trend towards higher plasma ET concentrations in ARAS and an elevated renal vein ET ratio ([Renal vein ET - inferior vena cava (IVC) ET] / IVC ET) on the stenotic side [245-247], others cannot demonstrate any significant difference in either systemic or renal vein ET concentrations in ARAS compared to essential hypertension or normal controls [248, 249]. Given the local nature of ET activity, receptor antagonist studies in man are required to establish a role for ET here; plasma ET probably does not have a role in diagnosis or prognosis of ARAS.

Pregnancy induced hypertension (PIH) and pre-eclampsia (PET)

With a few exceptions [250-252], plasma ET has repeatedly been demonstrated to be elevated in PIH/PET when compared to normal pregnancies of similar gestational age and to pregnant patients with pre-existing hypertension, the latter suggesting that the increased plasma ET is not due to a blood pressure effect per se [253-260] Also, while the elevated plasma ET concentrations do correlate negatively with creatinine clearance, they are probably not simply due to reduced renal clearance consequent upon renal dysfunction as elevated plasma ET concentrations are seen in PIH patients with normal renal function [261].

However, though elevated ET levels may correspond to disease severity, with a positive correlation between plasma ET and serum urate levels [255], and return to normal after parturition [253, 257-259] no clear relationship between ET levels and blood pressure has been demonstrated [256, 257]. PET is a disease characterised by vascular endothelial damage and thus the elevated ET levels might simply be a marker of generalised endothelial injury. However, increased ET-1 mRNA has been demonstrated in the placental villous tissue of pre-eclamptic pregnancies suggesting

an increased local production [262]. This could conceivably contribute to local blood flow redistribution and thus the placental ischaemia central to the pathogenesis of PET. A significant negative correlation exists between plasma ET levels and birth weight, low birth weight, in this respect, being a response to placental ischaemia [260, 263]. Thus, in respect of the hypertension of PET, the role of ET may be at the level of the placenta effecting a rise in systemic blood pressure by more general secondary mechanisms. Plasma ET does, however, appear to be a potentially useful correlate of disease severity in this condition.

Pulmonary hypertension (PHT)

ET-1 is elevated in experimental models of PHT [264] and in man, possibly consequent upon increased pulmonary production as the pulmonary venous concentration of ET-1 exceeds the arterial concentration [265]. Also, tissue levels of mRNA for ET-1 appear to correlate with the pulmonary vascular resistance [266]. Thus PHT, a disease characterised by endothelial injury, vascular smooth muscle cell proliferation and pulmonary vasoconstriction, is another potential target for ET receptor antagonists.

Non-selective and selective ET receptor antagonism are both able to prevent NG-monomethyl-L-arginine (L-NMMA) induced pulmonary vasoconstriction in a rat monocrotaline model of PHT [264]. The lack of effect of ETB antagonism suggests this is an ETA effect. In a pig hypoxic model, again, selective ETA and non-selective receptor antagonists were equally effective in countering hypoxia induced increases in pulmonary artery pressure with ETB selective drugs having no effect [267].

Consistent with these animal studies, two recent studies in 32 [268] and 213 patients [269] with PHT treated with bosentan over 12-16 weeks produced a clear improvement in clinical status. Similar initial encouraging results have been seen with the ETA receptor antagonist sitaxentan [270].

Systemic sclerosis

Systemic sclerosis is a disease characterised by vascular injury, fibrosis and, occasionally, malignant phase hypertension (hypertensive renal crisis) or pulmonary hypertension. While plasma levels of ET-1 are elevated in diffuse disease compared to limited cutaneous disease, there is no further rise in plasma levels in the subset of patients with diffuse disease who have hypertensive renal crisis or PHT [271, 272] suggesting that ET is not the trigger provoking transformation of the disease into these phases, nor is plasma ET a useful marker of the impending transformation. *In vitro* work has shown, however, that stimulated alveolar macrophages from scleroderma patients secrete higher amounts of ET-1 and big ET-1 than normal controls [273] and that there is a down regulation of ETA receptors in scleroderma fibroblasts [274]. ET is a powerful fibroblast and smooth muscle cell mitogen and its role systemic sclerosis may be a more central one in the pathogenesis of the disease with the elevated plasma levels representing an overproduction and subsequent overspill of this fibrotic mediator. Indeed, increased levels of ET-1 receptors have been demonstrated in scleroderma lung tissue, localised to the alveolar epithelium and the pulmonary interstitium, composed of mainly fibroblastic cells [275].

Additionally, the recent trials in PHT included patients with systemic sclerosis [268, 269], demonstrating clinical improvement in this group.

1.6.2b Congestive Heart Failure (CHF)

There is considerable evidence for a role of ET-1 in CHF. Experimentally, ET-1 causes hypertrophy of cardiac myocytes [276] and has a direct toxic effect on these cells [277]. In animal models of CHF, increased cardiac and pulmonary ET-1 synthesis has been documented [278-281]; In a Dahl salt-sensitive hypertensive rat model, the initial compensated left ventricular hypertrophy (LVH) was not associated with an increase in cardiac ET-1 but a five fold increase was noted after progression to the stage of left ventricular (LV) dilatation and global hypokinesis [282]. ETA receptors are more numerous than ETB receptors in both the normal and failing heart, but there may be an upregulation of ETA receptors in the failing heart [212, 278] suggesting that ETA receptors may be the major therapeutic target. As

with coronary artery disease, however, some evidence points toward the enhancement of vascular smooth muscle ETB mediated vasoconstriction in CHF [283-285]. Also, although an attenuation of myocyte contractility in response to ET-1 in cells from CHF animals and humans has been demonstrated [212, 286, 287], there is some evidence that endogenous ET-1 is involved in the maintenance of cardiac function in CHF [278], making cautious blockade of the ET system necessary.

Animal models of CHF have shown substantially improved survival with both selective ETA and non-selective ETA/B receptor antagonists. In CHF rats (coronary artery ligation model) although acute ETA receptor blockade with BQ-123 reduced myocardial contractility [279], long term treatment resulted in a 90% survival rate, compared to 40% for the non-treated animals [278]. This improved survival was associated with an amelioration of LV dysfunction and prevention of ventricular remodelling. Treatment with the non-selective receptor antagonist bosentan for 9 months in a coronary artery ligation model of CHF resulted in beneficial alterations in haemodynamics, cardiac geometry and function, and an 18% increase in survival in the treated group [288]. In the Dahl salt-sensitive hypertensive model of CHF, chronic treatment with bosentan, started at the stage of compensated LVH, did not alter LV mass but improved survival by 36% [282]. Of note, in a pacing CHF model, chronic (6 weeks) non-selective receptor antagonism also increased coronary sinus NO concentrations and improved endothelium-dependent relaxation in coronary arteries [289].

In man, the increase in circulating big ET-1 or ET-1 seen in patients with CHF correlates with the severity of symptoms and with prognosis [290, 291]. A series of trials point towards potential usefulness of ET receptor antagonists in this condition (Table 1.3), though it is unclear whether selective or non-selective receptor antagonism would offer the greatest benefit.

Initial studies with the non-selective ET receptor antagonist bosentan reported marked haemodynamic benefits both acutely and in the short term (2 weeks) [292,

293]. On the basis of these results, larger, longer-term trials have been undertaken with bosentan. The REACH study (370 patients followed for 6 months) was terminated early because of asymptomatic, reversible increases in hepatic transaminases, but reported improvements in symptoms, and reduced hospitalisations, in the treatment group in those completing the 6 month protocol [294]. However, in >1500 patients enrolled into the ENABLE study, bosentan conferred an early risk of worsening heart failure [295]. Acute studies with the combined ETA/B receptor antagonist tezosentan suggest that a much lower dose is required than originally to achieve benefit without significant side-effects [296-298].

The possible theoretical benefits of selective ETA antagonism in CHF have been explored in small acute studies. BQ-123 [299] and TBC 11251 [300] have produced significant systemic and pulmonary haemodynamic benefits respectively. Acute ETB receptor antagonism with BQ-788, caused systemic vasoconstriction in CHF patients, an effect reversed by the addition of BQ-123, suggesting that ETB receptors contribute to vasodilator tone in CHF [301]. The HEAT study demonstrated for the first time in a large patient population that 3 weeks of selective ETA receptor blockade improved CI in patients with CHF [302]. Larger, longer-term studies with ETA receptors have not, however, confirmed this potential. In a 24 week study, darusentan did not improve cardiac remodelling, clinical symptoms or outcome [303].

1.6.2c Coronary Artery Disease (CAD)

Ischaemia and myocardial infarction

Inducing coronary ischaemia in CAD patients results in a significant release of ET-1 of cardiac origin [304]. Plasma ET-1 concentrations have been shown to be high in CAD, particularly in patients with myocardial infarction or coronary vasospasm [305-308], and elevations of plasma ET-1 and big ET-1 in unstable angina and after myocardial infarction are correlated with a worse prognosis [309, 310] suggesting that an activated ET system may contribute to a poor outcome.

Both ETA and ETB receptors in the coronary circulation contribute to vasoconstriction [205, 311] and in isolated porcine and canine coronary arteries, TAK-044 inhibits ET-1 induced coronary vasoconstriction to a greater extent than BQ-123 [311]. These data might suggest that non-selective receptor blockade would be more appropriate than selective. In man, acute administration of the non-selective receptor antagonist bosentan to patients with CAD resulted in an increase in vessel diameter in coronary epicardial arteries, that was not further augmented by nitrates. This effect was, however, only clear in normal or mildly diseased arteries and was shown to correlate inversely with plasma LDL-cholesterol levels [312].

With respect to myocardial infarction, both ETA receptor and non-selective ETA/B receptor antagonists have been shown to reduce infarct size in coronary ligation models [313, 314]. Though these animal studies are promising, work on man in this respect has not yet been published.

Atherosclerosis

Plasma ET-1 concentrations are elevated in patients with atherosclerosis [239] and increased ET-1 had been demonstrated in atherosclerotic lesions [315] and throughout the vessel walls of patients with CAD [219] suggesting upregulation of the ET system in atherosclerosis.

In hypercholesterolaemic hamsters, ETA receptor inhibition decreases the progression of atherosclerosis mainly by reducing the number and size of macrophage-foam cells [316]. In addition, apolipoprotein E-deficient mice, genetically susceptible to severe atheroma and endothelial dysfunction, are protected from atherosclerosis when treated with ETA receptor antagonists and show an increase in NO-mediated endothelium-dependent relaxation [317]. Similarly, hypercholesterolaemic pigs treated with chronic ET receptor antagonists, both selective and non-selective, have improved coronary vascular function [318]. In respect of the failure of bosentan to significantly vasodilate severely diseased coronary arteries in man [312], it is possible that the lack of effect may relate to

greater endothelial dysfunction and that this might be respond to longer term ET receptor antagonism.

Post-angioplasty restenosis

ET-1 is known to have a mitogenic effect on vascular smooth muscle cells in vitro [319, 320]. In animal studies, expression of mRNA for all of the components of the ET system is increased at balloon angioplasty sites [321], and intra-arterial administration of ET-1 post-angioplasty increases neointima formation in vivo [322]. In humans, an increase in plasma ET-1 concentration is seen distal to the angioplasty site correlating with the duration and pressure of the balloon inflation [241, 323]. In animals, non-selective receptor antagonism with bosentan [324], SB 209670 [322, 325] or its analogue SB 217242 [326] reduces neointima formation after endothelial injury by balloon angioplasty whereas ETA receptor antagonism alone with BQ-123 is ineffective [325]. This may not indicate a need for combined receptor antagonism, however, as ETA receptor blockade with the potent, highly selective, oral agent ABT-627 does reduce restenosis [327]. Thus, targeting restenosis for prevention by ET antagonists might seem appropriate, although many other potentially useful agents have failed to fulfil their promise in this condition.

Arrhythmias

In animals, both combined receptor antagonism with SB 209670 and selective ETA receptor antagonism with LU 135 252 reduced the incidence of arrhythmias induced by ischaemia or exogenous ET-1 [328-330]. Of note, however, though BQ-123 did reduce ischaemic arrhythmias in rats at a low dose, a high dose increased mortality, due to an increase in refractory ventricular fibrillation [331].

1.7 Summary

ET receptor antagonists have been, and continue to be, crucial experimental tools in elucidating of the role of the ET system in renal and cardiovascular physiology and pathophysiology. Few studies have yet been performed in man to clarify the role of ET in renal function in health and disease, and on vascular function in chronic renal

disease. With the increasing availability of oral antagonists, both selective and non-selective, a knowledge of the actions of ET and its receptors is crucial to fully establish the clinical role of these drugs in renal and cardiovascular disease. If they live up to their promise, ET receptor antagonists could offer a wide range of benefits including a renoprotective effect, reduction of systemic and pulmonary blood pressure, a reduction of cardiac pre- and after-load, an improvement in coronary blood flow, and, in the longer term, a reduction in abnormal ventricular and vascular remodelling, and atherosclerosis. Through these effects, they might be useful in the management of CRF, hypertension, PHT, CAD and CHF.

1.8 Aims and Hypotheses

In a series of acute studies in healthy subjects and renal patients, this thesis explores the role of ET-1 and its receptors on systemic haemodynamics and renal function in health and renal disease.

Study 1: (Chapter 3) This study sought to clarify the role of ET-1 acting via the ETA receptor in the maintenance of normal vascular tone, hypothesising that previous systemic studies in healthy subjects with ET receptor antagonists have failed to demonstrate a haemodynamic effect as the doses of antagonists used have been insufficient to achieve significant ETA receptor blockade. The effect of incremental doses of ETA receptor antagonists to plasma concentrations sufficient to significantly block the ETA receptor was studied to ascertain a haemodynamic dose response curve for ETA receptor blockade. The information from this study was then used in the choice of ETA receptor antagonist dose in further studies.

Study 2: (Chapter 4) This study examined the interaction between angiotensin II (ANG II) and ETA receptor antagonism, hypothesising that the acute effects of ANG II would be mediated through the ETA receptor in healthy subjects.

Study 3: (Chapter 5) This study examined the effects of selective ETA and ETB, and combined ETA/B receptor antagonism on systemic and renal haemodynamics

and on renal tubular function in healthy subjects and patients with CRF. It explored the role of ET-1 in the maintenance of systemic and renal vascular tone in health and disease, and in renal tubular function. It also provided a direct comparison between selective ETA and combined ETA/B receptor antagonism. The hypothesis was that ETA receptor antagonism would produce vasodilatation, ETB receptor antagonism would produce vasoconstriction, and the addition of ETB to ETA receptor antagonism would attenuate the vasodilator effects of ETA blockade. It was also hypothesised that, based on forearm studies, the systemic haemodynamic response to ET receptor antagonism would be less in CRF patients compared to healthy controls, and finally that ETA receptor blockade should promote ET-1 induced, ETB receptor mediated natriuresis.

Study 4: (Chapter 6) This study examined the interaction between ET receptor antagonism and angiotensin converting enzyme (ACE) inhibition in healthy subjects. Based on animal data, the hypothesis was that there would be a synergism between these two agents.

Study 5: (Chapter 7) This study explored the mechanism of the synergy observed between ETA receptor antagonism and ACE inhibition in healthy subjects, hypothesising that this synergism would be mediated through NO, and possibly vasodilator prostaglandins.

Study 6: (Chapter 8) This study explored the actions of the ETB receptor by examining the effects of exogenous ET-1 in the presence of ETA receptor antagonism on systemic and renal haemodynamics and tubular function in healthy volunteers. The hypothesis was that, with the ETA receptor blocked, ET-1 would produce systemic and renal vasodilatation and natriuresis via the unblocked ETB receptor.

Study 7: (Chapter 9) This study examined the influence of a high salt diet on the effects of ETA receptor antagonism in healthy volunteers. The hypothesis was that,

in individuals whose blood pressure exhibited a high degree of salt sensitivity, the relative reduction in endothelial NO activity on a high salt diet would result in a reduced systemic haemodynamic response to BQ-123 compared to salt-resistant individuals.

Study 8: (Chapter 12) This study examined the effects of a single high dose of CyA on systemic and renal haemodynamics and tubular function in healthy volunteers. The purpose of this study was to establish a model of renal vasoconstriction that could then be used to explore the effects of intervention with ET receptor antagonists.

Study 9: (Chapter 13) This study sought to explain the systemic vasodilatation and lack of renal effects of high dose CyA in healthy volunteers by examining the differences in response to a standard dose of CyA in renal transplant patients chronically on the drug compared with those naïve to it. The hypothesis was that chronic exposure to CyA is required to produce the endothelial dysfunction, which then produces vasoconstriction to CyA.

Chapter 2

Materials and Methods

2 Methods

All studies were performed in the University of Edinburgh's Clinical Research Centre except for two patient studies (Chapter 5), which were performed in Monkland's Hospital, Airdrie, Lanarkshire, with the approval of the local research ethics committees and the written informed consent of each subject. The investigations conformed to the principles outlined in the Declaration of Helsinki.

All subjects abstained from alcohol, nicotine and caffeine-containing products for 24 hours, and had a light breakfast before attending on each study day. All studies were carried out in a quiet, temperature-controlled room, at 22-24^{0}C, with the subject recumbent throughout, except when voiding urine.

Healthy subjects taking any medications in the previous 2 weeks were excluded from the study. Patients continued taking their normal medication up to and including each study day with the exception of diuretics, which they omitted that morning. During renal clearance studies, (Chapter 5 - 13) subjects were asked to adhere to a standard diet containing 150 mmol sodium for 3 days before each study.

2.1 Drug administration

2.1.1 Locally active doses - intra-arterial administration

The brachial artery of the non-dominant arm was cannulated under local anaesthesia (1% lignocaine; Astra Pharmaceuticals, Stockholm, Sweden) with a 27 SWG steel needle (Cooper's Needle Works, Birmingham, UK) attached to a 16G epidural catheter (Portex Ltd, Hythe, Kent, UK), and patency was maintained by infusion of physiological saline (0.9%; Baxter Healthcare Ltd, Thetford, UK) at 1 ml/min. Saline was infused for 30 min prior to the infusion of ET-1.

2.1.2 Systemically active doses - intravenous administration

For systemic intravenous administration, study drugs were infused via an 18 SWG cannula sited in an antecubital vein. PAH and Inulin were diluted in dextrose (5%; Baxter Healthcare Ltd, Thetford, UK) and infused intravenously at a constant rate of

2 ml/min. All other drugs were dissolved in physiological saline and infused intravenously at a constant rate of 1 ml/min. Saline was administered as placebo.

2.2 Drugs

2.2.1 Endothelin-1

ET-1 (Clinalfa, Laufelfingen, Switzerland) was used as a non-selective ET receptor agonist. It is a 21 amino acid polypeptide that is highly selective for both the ETA and the ETB receptor (IC_{50}: ETA = 0.12 nM, ETB = 0.02 nM [96]) Its binding to its receptors is non-competitive.

In forearm studies (Chapter 3), ET-1 was dissolved in saline 0.9% and infused at a locally active dose of 5 pmol/min via the brachial artery for 90 min. This dose was selected from previous studies showing that ET-1 at this dose causes slow onset vasoconstriction of up to 40% in human resistance vessels [191].

In systemic studies (Chapter 8), it was infused at a dose of 2.5 ng/Kg/min for 60 min [118, 332]. The systemic effects of this dose are minimal [118]. The renal haemodynamic effects of this dose (-20% fall in RBF) return to baseline within 30 min of ceasing the infusion [118, 139] and are blocked by low dose BQ-123 [139].

2.2.2 BQ-123

BQ-123 (Clinalfa AG, Laufelfingen, Switzerland) was used as a selective ETA receptor antagonist (Chapters 3 - 11). It is a synthetic derivative of BE 18257B, a product of *Streptomyces misakiensisis* and is a cyclic pentapeptide that is highly selective for the ETA receptor (IC_{50}: ETA = 7.3 nM, ETB = 18 μM [93]). Studies with radiolabelled BQ-123 demonstrate that it binds competitively to the ETA receptor, achieving steady state within 7 minutes of injection and dissociates with a half-life of 1.4 min [94]. It is extracted from the circulation by the hepatic anion transport system [97]. It does not inhibit the binding of ANG II, calcitonin gene-related peptide or peptide YY to their respective receptors [93], and has been specifically shown not to bind to ANG II receptors [333].

In study 1 (Chapter 3), I sought to establish a dose response curve for BQ-123 in healthy humans. Doses ranged from 100 - 3000 nmol min^{-1} for 15 min. The starting dose was selected from a previous study investigating the local effects of BQ-123, which suggested that 100 nmol min^{-1} is the threshold around which systemic effects might be observed [191].

On the basis of this dose response curve, in subsequent studies (Chapter 4 - 11), a dose of 100 nmol/min for 15 min was used as a threshold dose for systemic ETA blockade, and 1000 nmol/min over 15 min as that achieving maximal haemodynamic effects. The haemodynamic effects of 1000 nmol/min for 15 min are demonstrable for ~2 hours after injection. BQ-123 is undetectable in the plasma 45 min after the 100 nmol/min dose and 150 min after the 1000 nmol/min dose.

2.2.3 BQ-788

BQ-788 (Clinalfa AG, Laufelfingen, Switzerland) was used as a selective ETB receptor antagonist (Chapters 5, 6, 10). It is a linear tripeptide that is highly selective for the ETB receptor (IC_{50}: ETA = 280 nM, ETB = 1.2 nM [96]). It is specific, (it does not inhibit the binding of ANG II, calcitonin gene-related peptide or peptide YY to their respective receptors [96]), and is a competitive inhibitor of the ETB receptor.

BQ-788 was infused at 30 and 300 nmol/min for 15 min, doses shown to be haemodynamically active in a previous systemic dose-ranging study [210].

2.2.4 Angiotensin II

ANG II (Clinalfa AG, Laufelfingen, Switzerland) (Chapter 4) was administered in ascending doses of 1, 3 and 6 ng/kg/min for 15 min at each dose. The doses were chosen from previously published studies [334, 335], and pilot studies in the department, to achieve a maximum increase in MAP of ~20 mmHg.

2.2.5 Noradrenaline

Noradrenaline (Sanofi Winthrop, Guildford, UK) (Chapter 4) ANG II (Clinalfa AG, Laufelfingen, Switzerland) was administered in ascending doses of 60, 120 and 210 ng/kg/min for 15 min at each dose as a control pressor agent. Doses of NE were selected from a previously published study [336], and confirmed by pilot studies in the department to give an equivalent pressor response to that achieved with the chosen doses of ANG II.

2.2.6 Enalapril

Enalapril (Dexcel Pharma Ltd, Daventry, UK) used as an ACE inhibitor (Chapter 6 - 8). It is a non-sulphydryl derivative of the amino acids L-proline and L-alanine. It reduces concentrations ANG II by inhibiting the conversion of ANG I to ANG II by ACE. ACE is also identical to kininase II that breaks down bradykinin. Some of the effects of ACE inhibitors may therefore be mediated by increased concentrations of bradykinin.

After oral dosing, ~60% of the dose of enalapril is absorbed, reaching peak plasma concentrations at 1 hour. It has low ACE inhibitory activity but is hydrolysed by liver esterases to form enalaprilat, the active drug (enalaprilat is poorly absorbed from the GI tract). Enalaprilat reaches peak plasma concentrations ~3-4 hours after administration of enalapril [337] and has a half-life of ~11 hours in the circulation. Excretion is primarily renal, and consequently delayed in renal failure. Steady state is achieved after 3-4 days administration in healthy volunteers [338].

Haemodynamically, enalapril causes an increase in arterial compliance, a reduction in systemic vascular resistance and a reduction in blood pressure. In healthy volunteers, RBF increases and renal vascular resistance falls without significant changes in GFR. [338]. Enalapril also produces a moderate increase in urinary sodium excretion 6-10 hours after oral administration [339]. In terms of hormonal effects, acutely, plasma renin activity increases while ANG II and aldosterone concentrations are reduced [340].

Plasma ACE inhibition is maximal between 2-10 hours after oral dosing, with 20 mg producing 80-95% ACE inhibition [339, 341, 342]. This ACE inhibition has been shown to be sustained over extended periods of up to 6 months of chronic dosing [338]. However, while a single dose of enalapril will still produce substantial ACE inhibition after 24 hours, ANG II concentrations have returned to baseline by this time [340]. Maximal increases in urinary sodium excretion, and renal haemodynamics do not occur until 72 hours of treatment [343].

Thus, I chose to use a dose of 20 mg twice daily for 5 days before each study because 20 mg ensures maximal achievable ACE inhibition, twice daily dosing ensures continued ANG II suppression, pre-treatment allows steady state to be reached and maximal renal changes to develop, and pre treatment does not result in an attenuation of ACE inhibition. The final dose of 20 mg was given at 0830 in each study day. Baseline measurements are therefore 2.5 - 3.5 hrs after administration, and the study measurements are made from 3.5 - 7.5 hr after administration. Thus, ACE inhibition was maximal throughout the period under study.

2.2.7 L-NMMA

L-N^G-monomethyl-arginine (L-NMMA) (Clinalfa AG, Laufelfingen, Switzerland) was used as an inhibitor of nitric oxide synthase (NOS) activity (Chapter 7). It is a specific substrate analogue that acts as a competitive inhibitor NOS in humans.

A dose of 3 mg/Kg over 5 min was used in the studies. Based on previous studies, this dose is sufficient to cause a small transient increase in blood pressure (7-10%) and decrease in RBF (-10%) with no adverse effects noted. After a 5 min infusion, blood pressure returns to baseline by 30 min and RBF returns to baseline by 60 min. The plasma half life of L-NMMA given in this form is 75 min [191, 193, 344].

2.2.8 Indomethacin

Indomethacin (Cox Pharmaceuticals, Devon, UK) is a cyclooxygenase (COX) inhibitor that prevents prostaglandin formation (Chapter 7). It was administered as a

single 100 mg dose at study start. This is sufficient to significantly reduce urinary prostaglandin formation [345]. Subjects were specifically instructed to eat breakfast on each study day, therefore, it has not been given on an empty stomach. None of the subjects recruited had dyspepsia or a history of peptic ulcer disease.

2.2.9 Slow sodium

Slow Sodium tablets (Novartis Pharmaceuticals, Frimley, UK), each containing 10 mmol of sodium, were used to supplement dietary sodium intake in high salt studies (Chapter 9).

2.2.10 PAH

Para-aminohippurate sodium (PAH, Clinalfa AG) was used for the measurement of renal plasma flow by standard clearance techniques [346] (Chapter 5 - 9, 12, 13). It is an inert and non-toxic compound that is both filtered at the glomerulus and actively secreted by the proximal tubules, reaching the kidney only via the blood stream. The extraction by the kidneys in a single transit is not complete (the full criteria for a marker of RBF by clearance) but about 80-90%, thus measurements are quoted as "effective" renal plasma flow (ERPF). This extraction rate is not affected by BQ-123, BQ-788 or ET-1 in man [141].

PAH was administered as a bolus loading dose of 0.4 g in 100 ml dextrose 5 over 15 min, and a maintenance infusion of 6.6 g/L at a rate of 2 ml/min. For subjects with a calculated GFR <40 ml/min, doses were reduced by a third.

2.2.11 Inulin

Inutest (Fresenius Pharma, Austria GmbH) was used for the measurement of GFR by standard clearance techniques [346] (Chapter 5 - 10, 12, 13). Inulin is an inert and non-toxic complex polyfructose with a molecular weight of 5,200 daltons. It is not protein bound, is freely filtered at the glomerulus, is neither secreted not reabsorbed within the tubules, nor metabolised within the kidney and hence fulfils the criteria for the measurement of GFR by clearance measurements. Its problems with

solubility have been overcome by the introduction of Sinistrin (Inutest) a related polysaccharide with identical clearance.

Inutest was administered as a bolus loading dose of 3.5 g in 100 ml dextrose 5 over 15 min, and a maintenance infusion of 10 g/L at a rate of 2 ml/min. For subjects with a calculated GFR <40 ml/min, doses were reduced by a third.

2.2.12 Cyclosporin

Cyclosporin Neoral (Novartis Pharmaceuticals, Frimley, UK) is a fungal peptide whose main target is the T lymphocyte, producing immunosuppressive activity. It is also known to have significant effects on blood pressure via actions on the sympathetic nervous system and the vascular endothelium and smooth muscle cells.

To study its vascular effects, healthy subjects CyA liquid, 10 mg/kg, or placebo (olive oil) administered in orange juice to disguise the taste (Chapter 12). This dose was chosen from a previous study demonstrating an acute, but transient decline in GFR after oral administration [347]. In patients, CyA was administered as a single dose of 100 mg (Chapter 13). This dose was chosen to be in line with standard doses taken by transplant patients on chronic therapy.

2.3 Haemodynamic measurements

2.3.1 Forearm blood flow

Blood flow was measured in both forearms by venous occlusion plethysmography using mercury-in-silastic gauges [348] that were securely applied to the widest part of each forearm. The hands were excluded from the circulation during each measurement period by inflation of a wrist cuff to 220 mm Hg. Upper arm cuffs were intermittently inflated to 40 mm Hg for 10 in every 15 sec to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. Recordings of forearm blood flow were made repeatedly at 10 min intervals over 3-minute periods. Voltage output from a dual-channel Vasculab SPG 16 strain gauge plethysmograph (Medasonics Inc) was transferred to a Macintosh

personal computer (Performa 475, Apple Computer Inc, Cupertino, CA, USA) using a MacLab analogue digital converter and Chart software (v. 3.2.8; both from AD Instruments, Castle Hill, NSW, Australia). Calibration was achieved using the internal standard of the Vasculab plethysmography units.

2.3.2 Blood pressure

Blood pressure was recorded in duplicate at each time-point using a well-validated semi-automated non-invasive oscillometric sphygmomanometer (Takeda UA-751 sphygmomanometer, Takeda Medical Inc) [349]. Recordings were required to be within 10 mmHg of each other (systolic and diastolic). If not, blood pressure was repeated until two consecutive readings did fulfil these criteria. During forearm studies (Chapter 3), blood pressure was recorded in the dominant arm (ie. not in the arm with intra-arterial cannulation.)

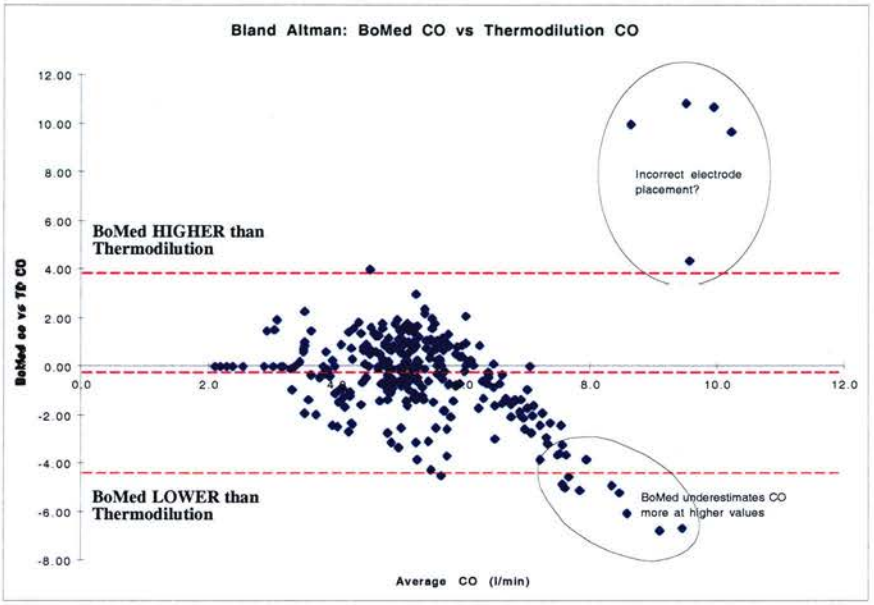
2.3.3 Cardiac output and heart rate

Cardiac output (CO, L/min) and heart rate (HR, bpm) were recorded using a well validated non-invasive bioimpedance technique (NCCOM3; BoMed Medical Manufacturer Ltd, Irvine, California, USA) [350, 351]. This non-invasive technique measures CO by transthoracic bioimpedance. A constant sinusoidal current is applied through dual electrodes situated at the root of the neck bilaterally and to the lateral aspect of the trunk at the level of the xiphisternum. These electrodes then detect changes in bioimpedance related to the cardiac cycle and blood flow. CO is estimated from the measures of bioimpedance by the Sramek-Bernstein formula, adapted from the original formula of Kubicek [351]. HR is counted directly from detection of the cardiac electrical cycle. Each reading is the average of 15 consecutive beats. Four such readings were recorded for each measurement of CI and HR.

Bioimpedance is useful for the measurement of changes in CO in an individual in acute interventional studies [352] and is best expressed as percentage change from baseline [351]. However, bioimpedance derived CO does not correlate well with CO measured by invasive methods. I have demonstrated, against thermodilution as a

gold standard, that it is least accurate at high CO values or when the % change from baseline is high. In these circumstances, it tends to underestimate the degree of change. As such it produces conservative estimates of changes in CO (Fig 2.1).

Fig 2.1 Comparison of cardiac output measured by thermodilution and bioimpedance



Data presented from a study performed in the Clinical Research Centre in patients with heart failure treated with endothelin antagonists (Study by S. Leslie). Subjects had CO measured by thermodilution during right heart catheterisation and by bioimpedance. Comparison of these two measures demonstrated good correlation over a wide range of CO. Bioimpedance did, however, underestimate high CO values when compared to Swan Ganz thermodilution measurements. A similar pattern was seen when change from baseline was compared. Again bioimpedance underestimated large changes in CO. Thus, in providing a conservative esitmate, bioimpedance is less likely to produce false positive results when examining changes in CO after vasoactive agents.

2.3.4 Clearance Studies

ERPF and GFR were measured by standard clearance techniques [346] (Chapter 5 - 10, 12, 13). On each study day, an 18 standard wire gauge (SWG) cannulae was sited in an antecubital vein in each arm. Diuresis was induced by 500 ml 5% dextrose over 30 min through the left arm cannula. After 15 min, the loading doses of PAH & inutest were administered through the same cannula. Thereafter, maintenance infusions of PAH and inutest, and 5% dextrose at 260 ml/hr continued throughout the study. Urine was collected by spontaneous voiding every 30 min. A two hour period was allowed for water, inutest and PAH equilibration before baseline measurements, made over two 30 min urine collection periods. Blood pressure, CO

and HR were recorded every 15 min. At the mid-point of each 30 min collection period, blood was sampled from the right antecubital cannula for PAH, inulin, and haematocrit measurements.

2.4 Assays

At pre-specified time points, venous blood was collected via an 18 SWG cannula for plasma measurements. Blood was collected into plain tubes (Sarstedt) for the measurement of serum sodium, and into EDTA tubes (Sarstedt) for all other plasma measurements. 20 ml aliquots of urine from each voiding were collected into plain tubes for the urinary measurements. At pre-specified time points, 20ml aliquots of urine were also collected into plain tubes containing 2.5ml of 50% acetic acid for the measurement of urinary ET-1.

Haematocrit (Hct) was measured on whole blood. All other blood samples were centrifuged immediately at 1000 *g* at 4^0C for 20 min, and plasma and urine stored in plain tubes at -80^0C until assay.

2.4.1 Plasma and urinary inulin

Inulin was determined by spectrophotometry after hydrolysis to fructose. Plasma samples were deproteinised with equal volumes of 6% perchloric acid and, after centrifugation at 1000 *g* for 10 min, supernatant was decanted. Urine was diluted 1/20 with 3% perchloric acid. Resorcinol (1.5 g dissolved in 1 l of ethanol) and HCl/$FeCl_3$ solution (7.5 mg $FeCl_3$ dissolved in 1 l of molar hydrochloric acid) were added in a 6:6:1 ratio to the plasma/urine. The samples were then vortexed and incubated at 80^oC for 40 min. Inulin concentrations were then determined against standard curves by absorption spectrophotometry at 480 nm.

2.4.2 Plasma and urinary PAH

PAH was determined by high performance liquid chromatography (HPLC) with fluorescence detection. Plasma samples were deproteinised with equal volumes of 6% perchloric acid and, after centrifugation at 1000 *g* for 10 min, supernatant was

diluted by 1/40 with deionised water. Urine samples were diluted by 1/4000. Dihydroxybenzylamine hydrobromide (DHBA) was used as an internal standard. Samples were injected into the HPLC column. The HPLC system consisted of a Waters 510 HPLC pump, WISP (Waters Intelligent Sample Processor) and Spherisorb S5 ODS column (Waters Ltd, Watford, Herts. UK) with detection by an LS-5 fluorometric detector (Perkin-Elmer Ltd, Beaconsfield, Bucks, UK), with excitation and emission wavelengths of 280 nm and 360 nm respectively. The mobile phase consisted of 0.1 molar citrate acetate buffer containing 100 mg/L octane sulphonic acid.

2.4.3 BQ-123

BQ-123 concentrations in plasma were measured by HPLC with fluorescence detection. One volume of plasma was precipitated with 4 volumes of ethanol, ultracentrifuged at 4°C for 15 min at 10,000 *g*, and the resulting supernatant injected into the HPLC column. The HPLC system consisted of a Waters 510 HPLC pump, WISP (Waters Intelligent Sample Processor) and Spherisorb S5 ODS column (Waters Ltd, Watford, Herts. UK) with detection by an LS-5 fluorometric detector (Perkin-Elmer Ltd, Beaconsfield, Bucks, UK), with excitation and emission wavelengths of 284 nm and 348 nm respectively. The mobile phase consisted of 60:40 acetonitrile: de-ionised water with tri-fluoroacetic acid at a concentration of 0.1%. The peptide TAK-044 was found to fluoresce at identical wavelengths to BQ-123 and was eluted from the column with a retention time similar to but not identical with BQ-123, allowing its separate measurement. Hence, TAK-044 was used as a standard in this assay. Recovery of BQ-123 from plasma was found to be 107% and the intra- and inter-assay variations were 5.8% and 9.6% respectively.

2.4.4 Haematocrit

Hct was measured using a Coulter counter.

2.4.5 Hormone measurements

After extraction with acetic acid using Bond ElutÆ columns (Varian, harbour City, CA, USA), [353] plasma ET-1 and ANG II concentrations were determined by standard radioimmunoassay (Peninsula Laboratories Europe, St Helens, UK), as previously described [354-356]. Plasma renin activity (PRA) was measured under standard conditions through the generation of ANG I as determined by radioimmunoassay [357]. Plasma aldosterone concentrations were measured by radioimmunoassay [358].

2.4.6 Serum ACE activity

Serum ACE activity was determined spectrophotometrically using a Sigma Diagnostics kit (Sigma Diagnostics St Louis, US). ACE hydrolyses the synthetic tripeptide N-[3-(2-furyl)acrylotl]-L-phenylalanylglygylglycine (FAPGG) to furylacryloylphenylalanine (FAP). This conversion results in a decrease in absorbance at 340 nm that can be calibrated against a standard sample to determine ACE activity. One unit of ACE activity is defined as that amount of enzyme that will catalyse the formation of one micromole of FAP from FAPGG per minute at 37°C. [359]

2.4.7 Plasma and urinary sodium

Urinary and plasma sodium concentrations were measured by flame photometry. Concentrations were determined against standard curves described by a quadratic equation. Plasma samples were diluted by 1/100 and compared against a standard curve ranging from 0 - 2 mmol/L. Urine samples were compared against a standard curve ranging from 0 - 40 mmol/L. Concentrated urine samples were diluted into this range as required.

2.4.8 Plasma and urinary osmolality

Urinary and plasma osmolality was measured by freezing point depression using a standard osmometer.

2.4.9 Urinary protein

Urinary albumin concentrations were measured using a double antibody technique (EURO/DPC Ltd, Llanberis, Gwynedd, UK) where ^{125}I labelled albumin competes with albumin in the sample for antibody sites. Separation of bound from free albumin is then achieved with a second antibody and the antibody bound fraction precipitated and radioactivity counted. Samples were compared against standard curves ranging from 5 - 60 μg/ml. Concentrated urine samples were diluted into this range as required. The assay has a detection limit of 0.3 μg/ml.

2.5 Data analysis

All results are expressed as mean ± standard error of the mean (SEM).

2.5.1 Forearm Blood Flow

Plethysmographic data listings were extracted from the chart data files and forearm blood flows calculated for individual venous occlusion cuff inflations using a template spreadsheet (Excel 4.0; Microsoft Ltd). As flow only stabilises after 60 sec of wrist cuff inflation, recordings made in the first 60 sec were not used for analysis. The last 5 flow recordings in each measurement period were calculated and averaged for the infused and non-infused arms [360]. To reduce the variability of blood flow data, the ratio of flows in the 2 arms was calculated for each time point, in effect using the non-infused arm as a contemporaneous control for the active treatment arm [348]. Forearm blood flow results are shown as the percentage change from basal values in the ratio of blood flow between infused and non-infused arm. Data were examined by repeated measures analysis of variance (ANOVA) and Bonferroni correction was applied to examine significance at each time point. Statistical significance was taken at the 5% level.

2.5.2 Systemic and Renal Data

Data were stored and analysed using the Microsoft Excel data analysis package (Excel 5.0, Microsoft Ltd). Blood pressure at each time point was calculated as the

mean of 2 recordings and represented as mean arterial pressure (MAP). Biompedance data at each time point were calculated as the mean of four recordings, each the average of 15 consecutive heart beats. Data were corrected using body surface area to give cardiac index (CI) for direct comparison of subjects, and systemic vascular resistance index (SVRI) derived from blood pressure and CI data (Table 2.1). GFR and ERPF were calculated from inulin and PAH clearances respectively. Effective renal blood flow (ERBF), effective renal vascular resistance (ERVR) and effective filtration fraction (EFF) were derived from these indices (Table 2.1). Urinary sodium excretion and fractional excretion were calculated from plasma and urinary sodium and inulin concentration and urinary flow rates.

Demographic data are expressed as mean ± standard error of the mean (SEM) and comparisons between groups examined by unpaired Student's *t*-tests. Baseline data were calculated as the mean of the 2 time points immediately preceding administration of the first study drug. Haemodynamic results are expressed as mean ± SEM placebo-corrected maximum (%) change from baseline. Statistical analysis was performed on untransformed data. Responses were examined by repeated measures analysis of variance (ANOVA) and Bonferroni correction was used to assess significance at specific time points. Area under the curve was calculated as a summary statistic of each curve and comparisons analysed by paired Student's *t*-tests. Statistical significance was taken at the 5% level.

Table 2.1 Calculations

Measurement	Calculation	Units
Mean Arterial Pressure (MAP)	$dBP + \frac{(sBP-dBP)}{3}$	mmHg
Systemic Vascular Resistance Index (SVRI)	$\frac{MAP \times 80}{CI}$	dyne.s /m^2/cm^5.
Glomerular Filtration Rate (GFR)	$\frac{UIn \times UFR}{PIn}$	ml/min
Effective Renal Plasma Flow (ERPF)	$\frac{UPAH \times UFR}{PPAH}$	ml/min
Effective Renal Blood Flow (ERBF)	$\frac{ERPF}{1\text{-}Hct}$	ml/min
Effective Renal Vascular Resistance (ERVR)	$\frac{MAP}{ERBF}$	mmHg.min/L
Effective Filtration Fraction (EFF)	$\frac{GFR \times 100\%.}{ERPF}$	%
Urinary Flow Rate (UFR)	$\frac{Urinary\ Volume}{Time\ of\ collection}$	ml/min
Urinary Sodium Excretion (UNaV)	UNa x UFR	(µmol/min)
Fractional Excretion of Sodium (FeNa)	$\frac{UNa \times PIn}{PNa \quad Uin}$	
Free water clearance (FWC)	$UFR \times (1 - \frac{UOsm}{POsm})$	ml/min

dBP - diastolic blood pressure, sBP - systolic blood pressure, U - urine, P - plasma, In - Inulin, PAH - para-amino hippuric acid, Hct - haematocrit, Na - sodium, Osm - osmolality

Chapter 3

Investigation of the effects of systemic ETA receptor antagonism in healthy volunteers

3.1 Introduction

Local studies in human forearm resistance vessels using phosphoramidon, an ECE inhibitor, and BQ-123, a selective ETA receptor antagonist, first demonstrated the importance of ET-1 in maintaining basal resistance vessel tone, in large part through an action on the ETA receptor [191]. These observations have since been confirmed by others [192, 194]. Responses in the forearm resistance vessels are usually predictive of those in the systemic circulation [360], so these data suggested that systemic ETA receptor antagonism would produce systemic vasodilatation. However, acute systemic administration of the selective ETA antagonist, BQ-123, was reported to have no effect on basal systemic haemodynamics [139, 199].

It is possible that a systemic haemodynamic effect of ETA receptor antagonism was not seen in these studies because the doses of BQ-123 used provided insufficient ETA receptor blockade to affect blood pressure. In addition, because healthy subjects have a number of reflex mechanisms that serve to defend blood pressure, it is possible that an important effect on systemic vascular resistance might have been missed by measurement of blood pressure alone. This chapter therefore describes two studies in healthy volunteers designed to address the issue of the role of the ETA receptor in the maintenance of vascular tone in healthy human subjects. The first study examines the haemodynamic effects of increasing doses of BQ-123, using bioimpedance cardiography to allow measurement of SVRI, with the aim of achieving a high degree of ETA selective receptor blockade. The second examines whether haemodynamically active doses of BQ-123 would antagonise the response to exogenous ET-1, by infusion of local doses of ET-1 into the forearm circulation, after administration of BQ-123 systemically, and measuring responses using forearm plethysmography.

3.2 Study design

3.2.1 Systemic haemodynamic study

This was a double-blind, placebo-controlled, balanced 5-way crossover study in 5 subjects, investigating the responses to four doses of BQ-123 (100, 300, 1000 and

3000 nmol/min for 15 min) and placebo. An ascending dose regimen was followed, allowing safety and tolerability of lower doses to be assessed before proceeding. Total doses of BQ-123 administered were 1.5, 4.5, 15 and 45 μmol (or 0.95, 2.84, 9.5 and 28.4 mg). The order of the placebo dose was randomly allocated so that each subject received it on a different visit (Table 3.1). Each visit was separated by at least 5 days. Subjects rested supine for 20 min, and baseline measures were then made in the 30 min before study drug administration. Haemodynamic measurements were made at 10 min intervals from 30 min pre-dose until 60 min post-dose, then at 30 min intervals until 2 h, then hourly until 4 h post-dose. Venous blood was obtained at 5, 15 and 240 min after BQ-123 infusion for measurement of plasma ET-1 and big ET-1. During the 300 and 1000 nmol/min phases, sub-aliquots of the samples were used for plasma BQ-123 assay.

Table 3.1 Systemic haemodynamic study randomisation

	Period 1	Period 2	Period 3	Period 4	Period 5
Volunteer 1	Placebo	100 nmol/min	300 nmol/min	1000 nmol/min	3000 nmol/min
Volunteer 2	100 nmol/min	Placebo	300 nmol/min	1000 nmol/min	3000 nmol/min
Volunteer 3	100 nmol/min	300 nmol/min	Placebo	1000 nmol/min	3000 nmol/min
Volunteer 4	100 nmol/min	300 nmol/min	1000 nmol/min	Placebo	3000 nmol/min
Volunteer 5	100 nmol/min	300 nmol/min	1000 nmol/min	3000 nmol/min	Placebo

Using MAP & SVRI measurements from a previous placebo-controlled study over 4 h [210], the study was calculated to have a power of ~90% to detect a 15% change in MAP and 20% change in SVRI (p=0.05) with 5 subjects.

3.2.2 ET-1 challenge study

This was a double-blind, placebo-controlled, 3-way crossover study in 5 subjects (3 of whom participated in the systemic study), investigating the effects of intra-arterial ET-1 on forearm blood flow (FBF), after treatment with either 300 or 1000 nmol/min of BQ-123 or placebo. After baseline infusion of saline for 30 min, subjects received a 15 min intravenous infusion of BQ-123 (300 or 1000 nmol/min) or placebo via a cannula in the right forearm, followed immediately by an intra-arterial infusion of ET-1 at a dose of 5 pmol/min for a total of 90 min via a left brachial artery cannula. Recordings of FBF by venous occlusion plethysmography were made repeatedly at 10 min intervals over 3-minute periods.

From FBF measurements in a previous study using ET-1 at 5 pmol/min [361], the study was calculated to have a power of 99% to detect abolition of the vasoconstriction response to ET-1 by BQ-123, and a power of ~80% to detect a 66% attenuation of this response ($p=0.05$) with 5 subjects.

3.3 Results

3.3.1 Systemic haemodynamic study

All 5 subjects (mean age 26 ± 2 yr, range 18 - 30 yr) completed all parts of the study. No adverse effects of treatment were reported.

Plasma ET-1 and big ET-1

Baseline values of plasma ET-1 and big ET-1 concentrations ranged from 4.4 to 5.2 pg/ml and 25 to 42 pg/ml respectively. There were no significant differences between baseline plasma ET-1 or big ET-1 concentrations in any phase of the study. Neither ET-1 nor big ET-1 changed significantly following treatment with any dose of BQ-123 or placebo (Table 3.2 & 3.3).

Plasma BQ-123 concentrations

Plasma concentrations of BQ-123 were undetectable with both doses at baseline. For 300 nmol/min, mean plasma concentrations were 126 ± 11 nmol/l at 5 min rising to

174 ± 20 nmol/l at 15 min. For 1000 nmol/min, they were 424 ± 33 nmol/l and 510 ± 64 nmol/l respectively (ETA receptor IC_{50} 7.3 nM; ETB receptor IC_{50} 18 μM) [93]. BQ-123 was no longer detectable in the plasma by 4 h at either dose.

Table 3.2 Plasma concentrations of ET-1 (pg/ml) after administration of BQ-123

BQ-123 (nmol/min)	Placebo	100	300	1000	3000
Baseline	4.91 ± 0.69	4.57 ± 0.48	5.23 ± 0.52	4.43 ± 0.28	2.88 ± 0.10
5 min	5.16 ± 0.74	4.08 ± 0.39	5.29 ± 0.49	6.07 ± 0.49	4.08 ± 0.49
15 min	5.81 ± 0.50	3.95 ± 0.65	5.49 ± 0.95	5.56 ± 0.95	4.03 ± 0.63
240 min	5.77 ± 0.75	4.44 ± 0.29	5.88 ± 0.65	5.80 ± 0.65	4.22 ± 0.41

Table 3.3 Plasma concentrations of big ET-1 (pg/ml) after administration of BQ-123

BQ-123 (nmol/min)	Placebo	100	300	1000	3000
Baseline	45.7 ± 14.4	37.8 ± 7.4	40.2 ± 6.5	57.5 ± 9.1	26.6 ± 6.7
5 min	49.3 ± 10.7	29.2 ± 1.9	38.5 ± 6.3	51.9 ± 8.5	34.7 ± 0.6
15 min	66.2 ± 14.4	26.0 ± 2.4	49.5 ± 14.1	51.4 ± 9.4	30.7 ± 3.1
240 min	38.8 ± 7.1	35.8 ± 5.2	48.4 ± 6.0	51.5 ± 9.2	30.1 ± 4.6

Haemodynamic parameters

Baseline measurements for haemodynamic parameters were similar during all treatment periods (Table 3.4). After BQ-123 administration, changes were apparent in all parameters by the first measurement at 10 min. Maximal changes occurred between 40 and 60 min, with a prolonged effect occurring at the 2 highest doses, excepting changes of HR, which were maximal at 15 min.

Table 3.4 Baseline haemodynamic measurements

BQ-123 (nmol/min)	**Placebo**	**100**	**300**	**1000**	**3000**	**ANOVA baseline data**
MAP (mmHg)	78.9 ± 1.5	79.2 ± 2.3	80.8 ± 3.5	78.2 ± 2.1	78.6 ± 3.3	p=0.96
SVRI (dyne.sec/cm^5/m^2)	1816 ± 104	1752 ± 136	1774 ± 144	1800 ± 232	1880 ± 176	p=0.98
CI (L/min/m^2)	3.52 ± 0.21	3.69 ± 0.25	3.80 ± 0.31	3.63 ± 0.38	3.45 ± 0.35	p=0.89
HR (bpm)	64.2 ± 6.6	57.1 ± 5.2	63.9 ± 6.6	54.5 ± 4.8	57.3 ± 5.9	p=0.69

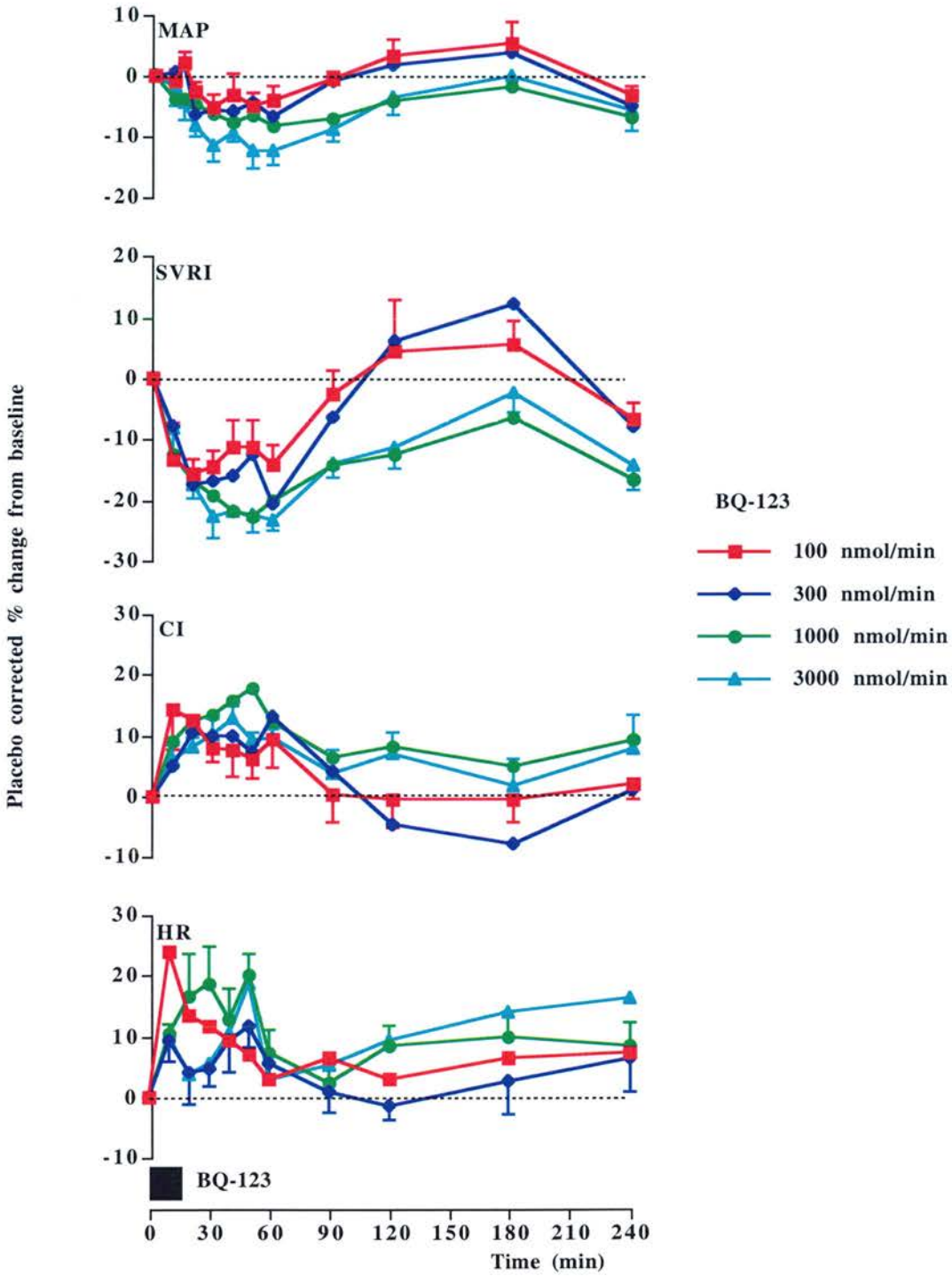
MAP decreased in a dose-dependent fashion. This was statistically significant at 300, 1000 and 3000 nmol/min BQ-123 (300 nmol/min: ANOVA $p<0.05$ vs. placebo, 1000 & 3000 nmol/min: $p<0.01$ vs. placebo) with a maximum mean placebo-corrected reduction of 12.4 ± 3.5% after 3000 nmol/min. Placebo corrected SVRI also decreased in a dose dependent fashion. This decrease was significant for all doses of BQ-123 (ANOVA $p<0.01$ vs. placebo). The maximum decrease in SVRI (23.3 ± 4.3%) occurred with 3000 nmol/min of BQ-123 (Table 3.5 & Fig 3.1). CI and HR increased significantly at all doses ($p<0.01$; ANOVA, Table 3.5 & Fig 3.1).

Table 3.5 Systemic haemodynamic responses to BQ-123

BQ-123 (nmol/min)	100	300	1000	3000
MAP (mmHg)	-4.8 ± 2.6% (-4.0 ± 2.5)	-6.8 ± 3.6% (-5.4 ± 2.8)	-8.2 ± 3.1%† (-6.4 ± 2.4)	-12.4 ± 3.5%† (-10.1 ± 3.2)
SVRI (dyne.sec/cm^5/m^2)	-15.8 ± 7.6% (-312 ± 168)	-20.6± 3.0%* (-360 ± 56)	-22.7 ± 5.2%† (-432 ± 152)	23.3 ± 4.3%† (-448 ± 208)
CI (L/min/m^2)	14.3 ± 8.9% (0.49 ± 0.32)	13.0 ± 2.2%* (0.47 ± 0.07)	17.9 ± 5.7%† (0.59 ± 0.16)	12.7 ± 1.6% (0.43 ± 0.04)
HR (bpm)	23.8 ± 13.4%† (12.1 ± 6.5)	11.8± 4.4% (6.8 ± 2.4)	20.0 ± 5.9%† (11.6 ± 3.0)	18.7 ± 6.8%* (10.7± 3.1)

Results given are maximum placebo corrected percentage change from baseline ± SEM * $p<0.05$ vs. placebo, † $p<0.01$ vs. placebo: ANOVA + Bonferroni correction

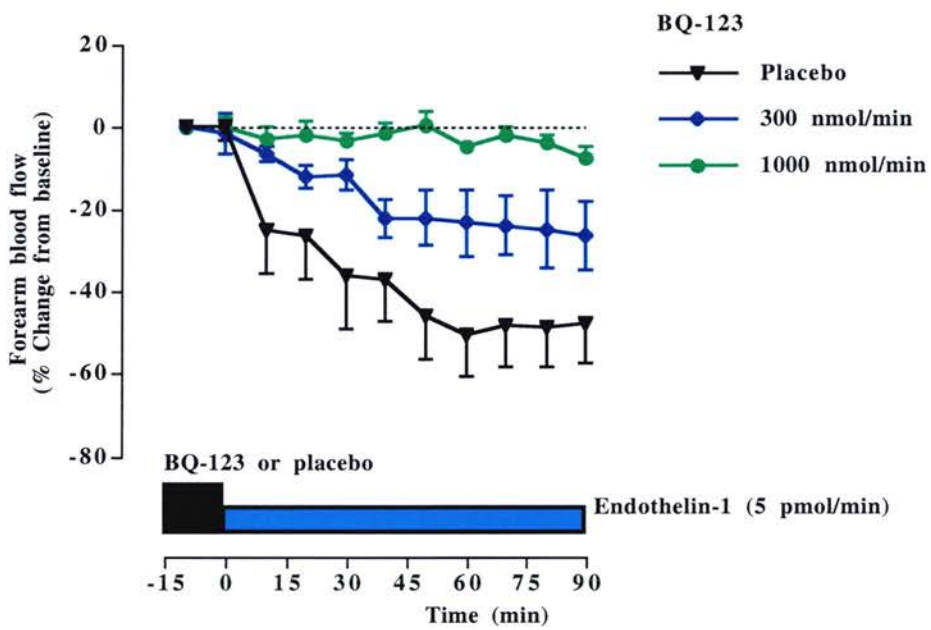
Figure 3.1 Systemic haemodynamics with ascending doses of BQ-123



3.3.2 ET-1 challenge study

Subjects who received placebo followed by local infusion of ET-1 developed a slow onset progressive vasoconstriction in the infused arm compared to the non-infused arm (maximum reduction in FBF: -48 ± 10% at 90 min). This response was attenuated by 300 nmol/min BQ-123 (-27 ± 8% at 90 min $p>0.5$ vs. placebo) and abolished by 1000 nmol/min BQ-123 (-8% ± 3%, $p<0.01$ vs. placebo, Fig 3.2).

Figure 3.2 Response in forearm blood flow to intra-arterial ET-1, when pre-treated with placebo or BQ-123 (300 or 1000 nmol/min for 15 min)



3.4 Discussion

This study demonstrates that, in healthy humans, BQ-123 causes substantial systemic vasodilatation, associated with a small but significant reduction in arterial blood pressure. A dose-dependent effect was observed, with little additional effect occurring above 1000 nmol/min. This work confirms the importance of the ET system, and of the vascular ETA receptor in controlling vascular tone and blood

pressure [36], and is in accord with the results of previous local forearm infusion studies [191, 192, 194].

There are several reasons for concluding that the effects on vascular tone and blood pressure are mediated by the ETA receptor. Measured BQ-123 concentrations in plasma at both 300 and 1000 nmol/min were substantially greater than the IC_{50} for BQ-123 at the ETA receptor. Even so, at 1000 nmol/min the plasma concentration of 510 nmol l^{-1}, was more than 35 fold lower than the IC_{50} for the ETB receptor (18 μM), consistent with effective but selective ETA receptor blockade [93]. In addition, there is a substantial body of evidence that the ETB receptor is a clearance receptor for ET-1 [362-364] and that agents that block the ETB receptor in vivo cause increases in plasma ET-1 concentrations [201, 202, 210, 293]. In contrast, in this study, there was no significant increase in either big ET or ET-1 plasma concentration at any dose of BQ-123. Finally, it has previously been shown that the net effect of systemic ETB receptor blockade is to cause systemic vasoconstriction [210], which would suggest that any ETB blockade would attenuate the vasodilatation associated with ETA blockade. Indeed, this effect may contribute to the lack of further vasodilatation at the highest dose of BQ-123, which was associated with a tendency for a rise in plasma ET-1 concentration, also consistent with a threshold effect on the ETB receptor at this dose.

Doses sufficient to lower blood pressure (300 & 1000 nmol/min) also antagonised the forearm vasoconstriction to brachial artery administration of ET-1 and, in keeping with its submaximal effect on SVRI, the lower dose of BQ-123 (300 nmol/min) only partially antagonised forearm vasoconstriction to ET-1. Of note, however, in the presence of a higher degree of ETA blockade, exogenous ET-1 failed to produce vasodilatation via the unblocked ETB receptor. This possibly reflects the local balance of dilator and constrictor effects mediated by endothelial and vascular smooth muscle ETB receptors. However it is also possible that the locally administered ET-1 was washed out by an increase in forearm blood flow consequent upon the vasodilatation induced by systemic BQ-123. Comparison with a

constrictor agent unaffected by ET-A antagonism would be needed to clarify this further.

Previous studies in healthy men [139, 199] failed to demonstrate a significant effect of BQ-123 on basal haemodynamics. However, the doses used were substantially lower, at 23.7 nmol/min for 60 min, followed by 94.8 nmol/min for 60 min [139] and ~9 nmol/min for 90 min [199]. These should be compared with 100 nmol/min BQ-123 for 15 min as the threshold dose for a systemic haemodynamic effect in our studies. In addition, both other studies measured blood pressure but not systemic vascular resistance, whereas, from the current study, the latter was a more powerful measure of the vascular effect of BQ-123, underlining the importance of this measurement in detecting modest haemodynamic influences. In this regard, other published studies in humans with ET antagonists do appear to show modest (~10 mmHg) reductions in blood pressure, with both bosentan (mixed ETA/ETB) [202] and ABT-627 (ETA selective) [200]. In the latter study, although systemic haemodynamics were only recorded at 30 min and 8 hours after dosing, systemic vascular resistance was measured and significant effects on this parameter were found in both acute and chronic dosing.

It should be noted that our research group has also previously failed to detect significant systemic effects, as measured by blood pressure and HR, at our lowest dose of BQ-123 (100 nmol/min) when administered into the brachial artery [191]. These current results suggest that 100 nmol/min does have a small systemic action, most apparent in its effects on systemic vascular resistance, that was not detected in forearm studies, perhaps because the major vasodilatation is in other vascular beds. In recognition of this potential problem, our research group has more recently used a 10-fold lower dose of 10 nmol/min BQ-123 [194], as a local dose for forearm studies. The study confirms the rationale for this approach.

It is also interesting to note that a 15 min infusion of BQ-123 produces haemodynamic effects for up to 4 hours at the higher doses. Although, in this study, there are measurements of BQ-123 concentrations only at 0, 15 and 240 min,

subsequent experiments, with identical dosing schedules of BQ-123, demonstrate that the peak concentration is achieved at the end of the infusion, falls to ~10% by 30 min and is undetectable by 75 min post infusion (Studies 3 & 4, Chapters 5 & 6). This suggests that the observed responses are a pharmacodynamic effect rather that a reflection of the plasma half-life of BQ-123. This is similar to our group's experience with the non-selective ET antagonist TAK 044 where the systemic haemodynamic effects of a 15 min bolus were still observable at 24 hours, whereas the peptide had a plasma half-life of 30-60 min [201].

In the current study, there was an increase in HR similar to that observed in other acute studies [202, 312]. These effects are not generally seen in chronic dosing studies with ET antagonists in patients with either hypertension or heart failure [224, 293]. For this reason, the effects are probably mediated through the activation of a cardiopulmonary reflex response to systemic vasodilatation rather a direct chronotropic effect on the heart.

Although the total number of subjects studied was low (n=5), the power of the study was sufficient to allow clear conclusions to be drawn. Given the limited experience with BQ-123 at these systemic doses, there were safety reasons for keeping the number of subjects to a minimum. In this regard, it is reassuring to note that, despite substantial systemic vasodilatation, and significant lowering of the mean arterial pressure, no side effects were observed or reported by the subjects.

In conclusion, this study with BQ-123 demonstrates that systemic ETA receptor antagonism causes substantial peripheral vasodilatation and modest lowering of blood pressure, consistent with an important role for the ET system in the maintenance of vascular tone in man.

Chapter 4

Actions of ETA receptor antagonism on the pressor response to angiotensin II in healthy volunteers

4.1 Introduction

ANG II is a powerful vasoconstrictor substance formed from its inactive precursor ANG I by the actions of the endothelial cell ecto-enzyme, ACE [365]. The success of ACE inhibitors in treating conditions such as hypertension and congestive heart failure underlines the importance of ANG II in pathophysiological situations involving vasoconstriction [366-368]. Recently, several lines of evidence have suggested that the effects of ANG II on vascular structure and function are at least in part mediated by ET-1. Studies in rats have demonstrated that chronic administration of, [369-373] or pre-treatment with [374] ET receptor antagonists can attenuate [370-372] or even abolish [369, 373, 374] the pressor response to chronic ANG II infusion. In contrast, acute administration of ET receptor antagonists show no effect on, [375, 376] or at best only partial blunting of [377-379] the pressor response to acute ANG II infusions.

This study investigates the effects of acute ETA receptor blockade with BQ-123 on the acute pressor response to ANG II in man, using a dose of BQ-123 known to have maximal haemodynamic actions and to block the vasoconstrictor effects of exogenous ET-1 (Chapter 3). To allow for any non-specific hypotensive and vasodilatory effects of BQ-123 on the response to ANG II, norepinephrine (NE) was used as a control pressor agent.

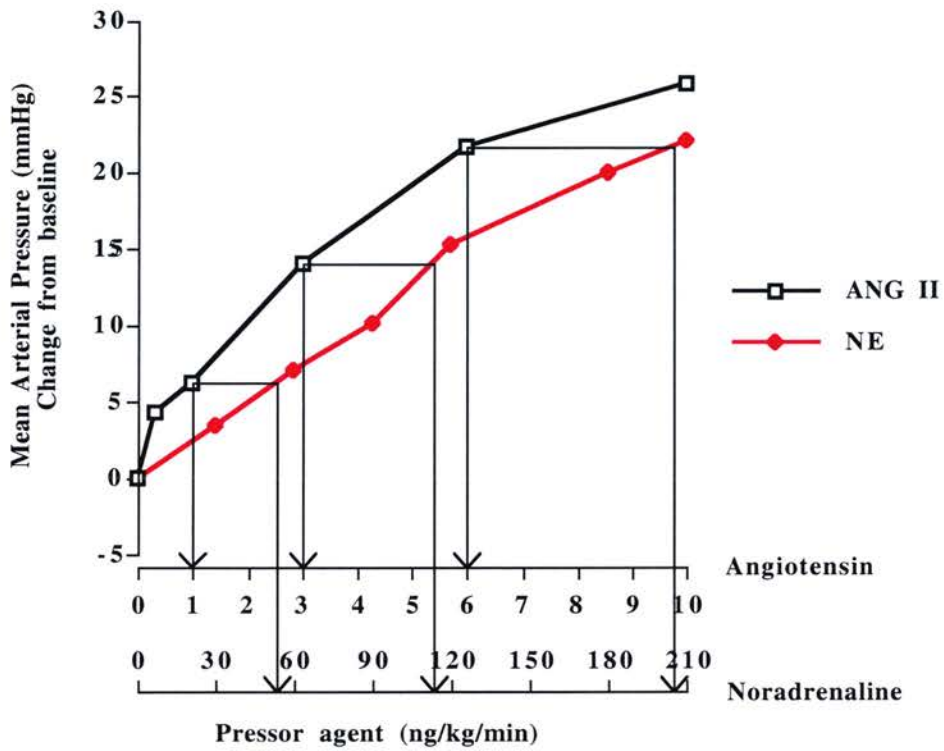
4.2 Study design

This was a double-blind, placebo-controlled study in eight subjects, who attended for a total of 4 visits. On two study days they received ANG II, and on two NE. The subjects were randomly assigned to receive ANG II or NE first and, for each pressor agent, subjects received BQ-123 or placebo in random order. A pilot study was performed in 2 subjects receiving ANG II and NE in incremental doses to confirm the equivalent pressor effect of the chosen study doses of these agents (Fig 4.1).

On each study day, subjects rested supine for 15 min before beginning the study. Saline was then infused intravenously for 30 min, followed by BQ-123 or placebo

for 15 min, then saline for a further 15 min. ANG II or NE was then administered in three incremental ascending doses for 15 min at each dose. The timing of the pressor infusions was chosen to correspond to the period of maximal vasodilatation, and hence inhibition of ETA receptor function, to this dose of BQ-123 (Chapter 3). Blood pressure and HR and CI were recorded in duplicate at 15 min intervals from 45 min before administration of BQ-123 or placebo until completion of the ANG II or NE infusions. Blood was taken from the left forearm cannula for the measurement of ET-1 and ANG II before administration of BQ-123/placebo, at 30 min after BQ-123/placebo infusion and after each dose increment of ANG II/NE.

Figure 4.1 Pilot study (n=2 subjects) to confirm equivalence of pressor effect



Doses for study: ANG II - 1, 3 and 6 ng/kg/min for 15 min at each dose, NE - 60, 120 and 210 ng/kg/min for 15 min at each dose.

4.3 Results

All 8 subjects (age 28 ± 4 yr, body mass index 24.6 ±1.0 kg/m^2) completed all parts of the study. No adverse events were reported.

4.3.1 Haemodynamic parameters

Baseline haemodynamic measurements were similar on all four study days (Table 4.1).

Table 4.1 Baseline haemodynamic data

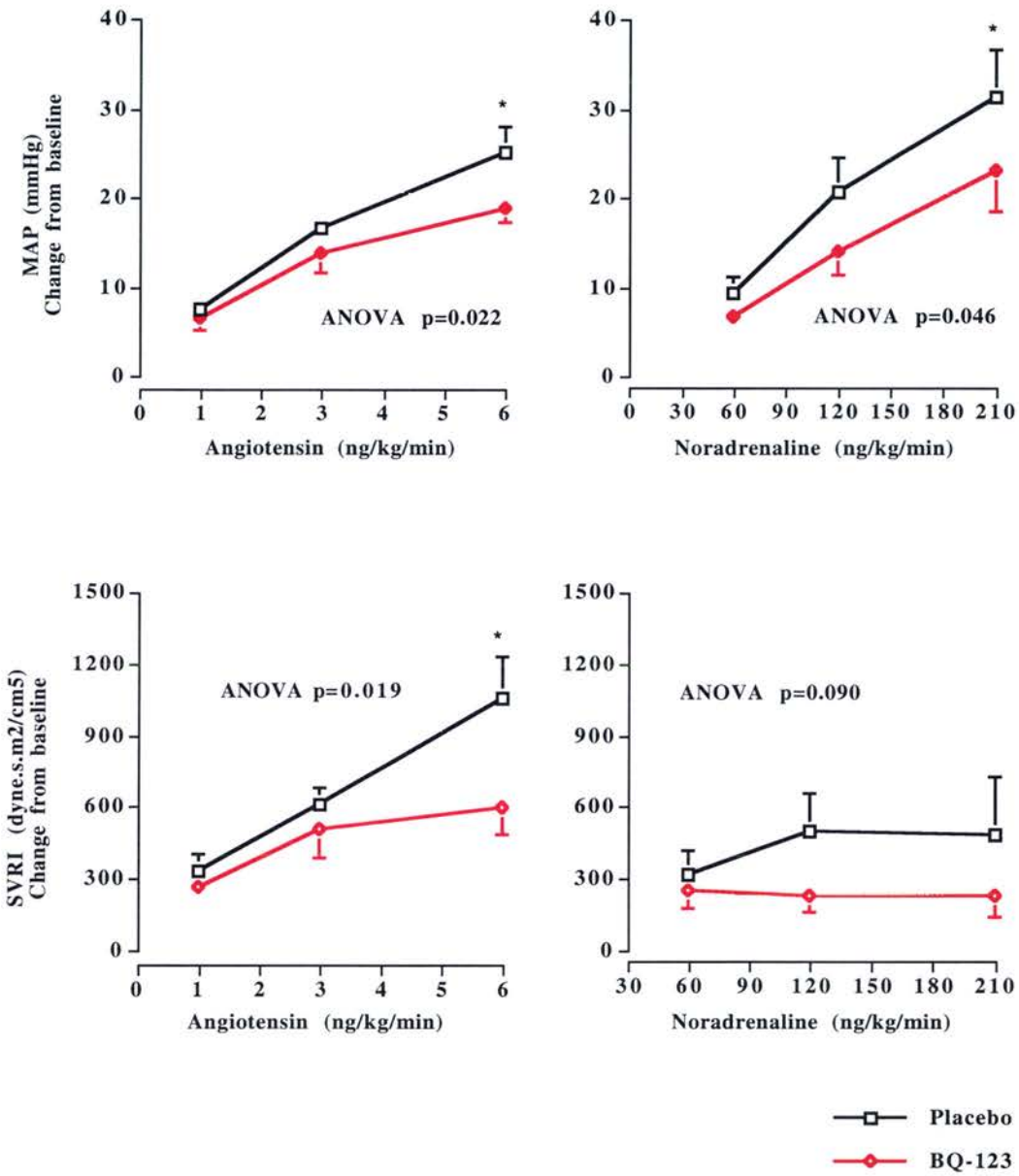
	ANG II + Placebo	ANG II + BQ-123	NE + Placebo	NE + BQ-123	ANOVA
MAP (mmHg)	81.5 ± 2.5	83.5 ± 2.5	84.5 ± 2.8	83.1 ± 3.5	p=0.897
SVRI (dyne.s /m^2/cm^5)	1712 ± 240	1736 ± 248	1744 ± 256	1792 ± 272	p=0.997
HR (bpm)	60.3 ± 1.8	61.3 ± 2.3	65.7 ± 1.9	63 ± 1.8	p=0.279
CI (L/min/m^2)	4.3 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.1 ± 0.3	p=0.986

After pre-treatment with placebo, ANG II and NE both increased blood pressure in a dose-related manner and to a similar extent (maximum increase in MAP: ANG II+placebo: 25.3 ± 2.8 mmHg, NE+placebo: 31.4 ± 5.1 mmHg, $p<0.01$ vs. baseline for ANG II and NE, p=0.12, ANG II vs. NE), though the mechanism of the pressor effect appeared different for ANG II and NE. The increase in MAP with ANG II was associated mainly with an increase in SVRI, whereas NE significantly increased CI, as well as SVRI.

Administration of BQ-123 was associated with a small reduction in MAP at 30 min and a subsequent significant attenuation of the observed pressor effect of both ANG II and NE (maximum response to ANG II+BQ-123: 18.8 ± 1.5 mmHg, p=0.02 vs.

placebo, NE+BQ-123: 23.0 ± 4.7 mmHg, p=0.04 vs. placebo). This attenuation was of a similar magnitude for both agents (*P*=0.28) (Fig 4.2). BQ-123 reduced SVRI compared to placebo (% change from baseline at 30 min; BQ-123: -3.1 ± 2.8%, Placebo: +4.9 ± 1.6%, p=0.02). The increase in SVRI observed with the pressor

Figure 4.2 Haemodynamic response to ANG II and NE ± BQ-123



agents was also significantly attenuated by BQ-123 in the case of ANG II (-5.7 ± 2.0: p=0.019) but not for NE (-3.2 ± 2.1: p=0.090) (Fig 4.2). However, the magnitude of the BQ-123 effect was not significantly different for the two agents (p=0.875). HR decreased during both ANG II and NE infusions (maximum reduction ANG II+placebo: -3.1 ± 2.1 bpm, NE+placebo: -11.0 ± 3.3 bpm), and was not altered by pre-treatment with BQ-123 (maximum reduction ANG II+BQ-123: -2.6 ± 1.9 bpm, p=0.57 vs. placebo, NE+BQ-123: -9.9 ± 3.4 bpm, p=0.13 vs. placebo).

4.3.2 Plasma ET-1 and ANG II

Plasma concentrations of ET-1 were not affected by ANG II or NE infusion at any time point (Table 4.2). Plasma ANG II concentrations increased with each successive ANG II dose increment, but there were no significant differences in the plasma ANG II levels between the BQ-123 and placebo phases (Table 4.3).

Table 4.2 Plasma ET-1 concentrations (pg/ml)

	ANG II + Placebo	**ANG II + BQ-123**	**NE + Placebo**	**NE + BQ-123**
Baseline	2.41 ± 0.35	3.81 ± 0.51	2.72 ± 0.41	2.32 ± 0.23
30 min post BQ-123	3.07 ± 0.12	3.05 ± 0.28	2.67 ± 0.55	2.40 ± 0.44
Post ANG II (1 ng/Kg/min)/ **NE** (60 ng/Kg/min)	2.74 ± 0.42	3.47 ± 0.49	2.88 ± 0.38	3.06 ± 0.28
Post ANG II (3 ng/Kg/min)/ **NE** (120 ng/Kg/min)	3.10 ± 0.27	3.21 ± 0.33	3.38 ± 0.68	3.13 ± 0.43
Post ANG II (6 ng/Kg/min)/ **NE** (210 ng/Kg/min)	3.04 ± 0.26	3.60 ± 0.43	3.89 ± 0.79	3.12 ± 0.44

Table 4.3 Plasma ANG II concentrations (pg/ml)

	ANG II + Placebo	ANG II + BQ-123
Baseline	21.8 ± 7.1	12.0 ± 1.6
30 min post BQ-123	23.4 ± 8.6	11.8 ± 2.2
Post ANG II (1 ng/Kg/min)/ **NE** (60 ng/Kg/min)	34.7 ± 4.5	27.2 ± 4.7
Post ANG II (3 ng/Kg/min)/ **NE** (120 ng/Kg/min)	67.8 ± 8.5	83.2 ± 20.4
Post ANG II (6 ng/Kg/min)/ **NE** (210 ng/Kg/min)	97.8 ± 9.5	121.6 ± 20.8

4.4 Discussion

These studies demonstrate that the blunting effect of acute ETA antagonism on the pressor actions of ANG II in man is modest and non-specific, having a similar effect on the pressor response to NE. This response is similar to that seen with BQ-123 alone (Chapter 3). This suggests that ET-1, acting through the ETA receptor, does not contribute importantly to the acute pressor response to ANG II in man.

Animal evidence suggests that ANG II can interact with the ET system. ANG II stimulates ET-1 gene transcription [21, 22, 380] and peptide secretion [33, 381] *in vitro* in a variety of cell types, including endothelial and vascular smooth muscle cells, probably via the AT-1 receptor [382]. *In vivo*, (Table 4.4) a series of studies in rats have demonstrated that pre-treatment with, or concomitant administration of, either selective ETA [374] or combined ETA/B [369, 373] receptor antagonists can abolish the pressor effects of chronically administered ANG II, although this effect is blunted [370-372] or lost [383] at higher ANG II doses. By contrast, after acute administration of ANG II, concomitant administration of both selective ETA [379]

and combined ETA/B [375, 377-379] antagonists have demonstrated either no effect on, [375] or at best only partial blunting of [377-379], the pressor response.

Table 4.4 Animal *in vivo* studies ANG II + ET receptor antagonism

Study	ANG II dosing Acute/Chronic	Dose	ET receptor antagonist	ET receptor antagonist dosing Acute/Chronic	Outcome on pressor response
Gardiner 1999[375]	Acute (bolus)	1.25-125 pmol/kg	SB 209670	Acute	No effect
Boemke 2001[376]	Acute	4 & 20 ng/Kg/min	LU 135252	Acute	No effect
Balakrishnan 1996[377]	Acute	3,9,30,90 ng/Kg/min	Bosentan	Acute	Partial blunting lower doses only
Heinemann 1997[378]	Acute (bolus)	0.1 – 3 nmol/Kg	Bosentan	Acute	Partial blunting
Riggleman 2001[379]	Acute	0.5 & 2 ng/Kg/min	BQ-123	Acute	Partial blunting
			Bosentan	Acute	Partial blunting
Casellas 1997[383]	Chronic	400 ng/Kg/min	Bosentan	Chronic	No effect
D'Uscio 1997[370]	Chronic	200 ng/Kg/min	LU 135252	Chronic	Partial blunting
Moreau 1997[371]	Chronic	200 ng/Kg/min	LU 135252	Chronic	Partial blunting
Barton 1998[372]	Chronic	200 ng/Kg/min	LU 135252	Chronic	Partial blunting
Herizi 1998[369]	Chronic	200 ng/Kg/min	Bosentan	Chronic	Abolished
Ortiz 2001[373]	Chronic	5 ng/Kg/min	Bosentan	Chronic	Abolished
Alexander 2001[374]	Chronic	50 ng/Kg/min	ABT-627	Pre-treatment	Abolished

Indeed, in dogs, selective ETA antagonism has been shown to have no effects on the rise in blood pressure during acutely administered ANG II at doses of the pressor agent ten-fold lower than those used in chronic studies [376]. However, it should be

noted that none of these studies included an ET-independent pressor agent as a control, and thus it is possible that any effects observed could be non-specific, relating to the direct vasodilatory properties of ETA and ETA/B receptor antagonists.

This interaction was explored in this study using the ETA selective receptor antagonist, BQ-123, because of evidence from systemic studies supporting the role of ETA receptors in the maintenance of vasoconstrictor tone [194] (Chapter 3) and of ETB receptors in vasodilator tone [194, 210]. Study 1 (Chapter 3) has shown that the dose of BQ-123 used in this study, 1000 nmol/min for 15 min, gives maximal functional ETA receptor blockade in healthy volunteers. As this earlier study has demonstrated that this dose of BQ-123 has a significant effect on blood pressure and systemic vascular resistance, confirmed in this study by a reduction in SVRI 15 min after the end of the BQ-123 infusion of ~8% compared to placebo, a control pressor agent was used to identify any non-specific hypotensive and vasodilatory effects of BQ-123 at this dose. NE was used as a control pressor agent because previous animal studies have shown that, while ET receptor antagonists can reduce ventricular hypertrophy induced by chronic NE infusion [384], in the acute setting, while inhibiting ANG II induced aortic ring contractions, they do not affect NE induced contractions [333]. Additionally, while ET and ANG II have been shown to act synergistically to increase blood pressure *in vivo*, this is not so for the combination of ET-1 and NE [385]. These data tend to support the use of NE as an ET-independent pressor agent in this acute study. As such, the similar efficacy of BQ-123 in attenuating equivalent rises in blood pressure induced by ANG II and NE suggests that the modest effect of acute ETA blockade on the pressor actions of ANG II is non-specific. Wenzel et al have recently demonstrated that BQ-123 can attenuate both ANG II and NE induced vasoconstriction in the skin microcirculation of healthy volunteers and postulate an acute ET-1 interaction with both ANG I and NE [386]. As with this study, however, the dose of BQ-123 used itself produced vasodilatation, and as such, it is equally likely that the attenuation of ANG II or NE induced vasoconstriction seen after ETA receptor inhibition is non-specific. Additionally, although the essentially paracrine nature of the ET system may makes

interpretations of changes in plasma ET-1 concentrations difficult, the failure of ANG II or NE to increase plasma ET-1 concentrations in this study also suggests that neither ANG II nor NE achieve their acute pressor effect by stimulating ET-1 generation. However, it is possible that ET-1 secretion may occur with chronic elevation of plasma ANG II and that this may influence vascular structure rather than function.

In conclusion, this study demonstrates a modest and non-specific inhibition by BQ-123 of the pressor response to ANG II in man suggesting that ANG II does not achieve its acute pressor effect via ET-1. It must be noted that this study looks only at acute effects, and further chronic interaction studies are needed in man, particularly in relation to vessel structure. Nevertheless, the lack of an acute ETA-ANG II interaction on vascular tone is consistent with clinical studies showing an added haemodynamic benefit of both selective ET_A and non-selective $ET_{A/B}$ in patients with heart failure already treated with ACE inhibitors [293, 299]. This supports the development of ET receptor antagonists as adjunctive treatments to ACE inhibitors.

Chapter 5

Actions of ET-A and ET-B receptor antagonism on systemic and renal haemodynamics and tubular function in hypertensive patients with chronic renal failure and healthy controls

5.1 Introduction

Previous ET receptor antagonist studies in man have failed to demonstrate any role for ET-1 in the maintenance of normal renal vascular tone [120, 139-141, 199]. To date, the effects of ET receptor antagonists on either basal systemic or renal haemodynamics in CRF have not been investigated. CRF is characterised by systemic and renal vasoconstriction, and commonly associated with hypertension. Given that ET receptor antagonists cause vasodilatation [191, 201], lower blood pressure [201, 224], and also ameliorate renal dysfunction in experimental models of kidney disease [387], they may be useful in the treatment of CRF.

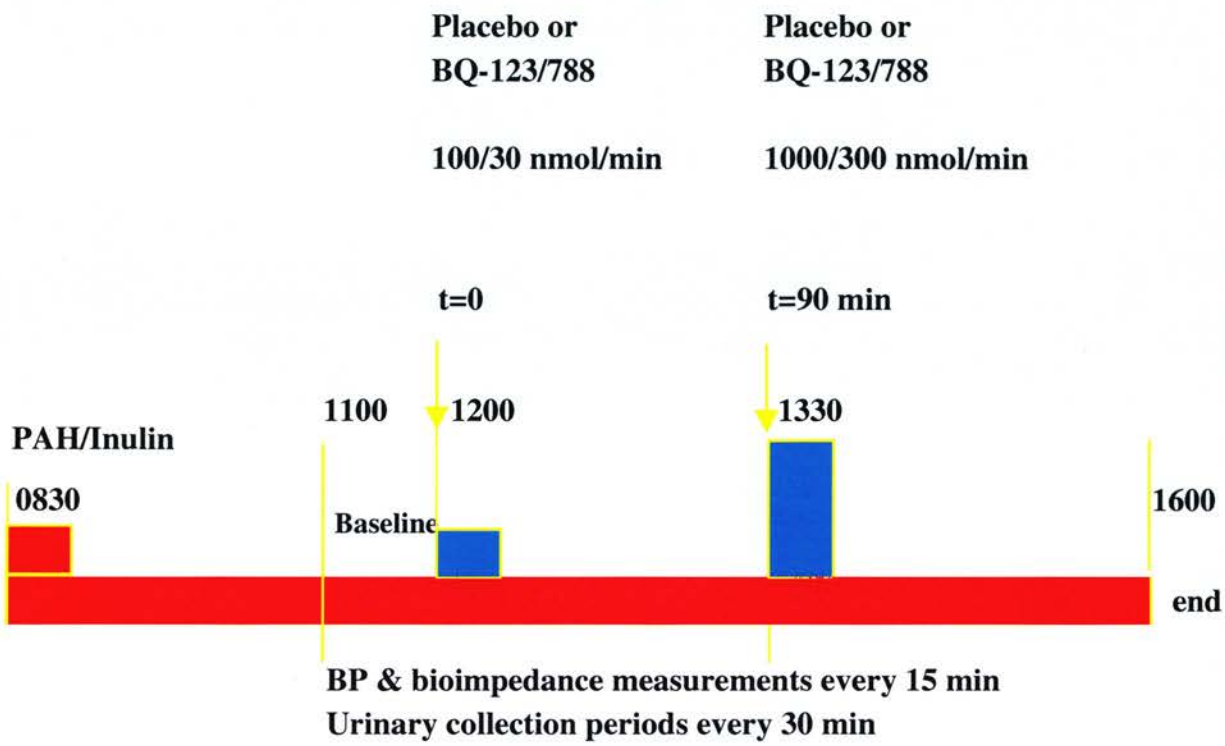
The first aim of this study was to investigate whether ET receptor antagonists at doses known to be maximally systemically active (Study 1, Chapter 3) would have effects in the renal circulation in healthy volunteers. Secondly, it investigates whether ET receptor blockade can produce alterations in systemic or renal haemodynamics in CRF. Because of the potential opposing effects at ETA and ETB receptors, and to establish which pattern of ET receptor blockade may be the more beneficial therapeutic combination, this study also directly compared the effects of regimens involving ETA and ETB receptor blockade separately, and in combination in CRF patients and matched healthy controls.

5.2 Study design

This was a randomised, double-blind, placebo-controlled study in 8 patients with CRF and 8 matched healthy controls. Subjects attended for 4 visits, each separated by $\geq$7 days, receiving placebo, BQ-123, BQ-788 or the combination of BQ-123 and BQ-788. Four comparisons of interest were pre-identified as placebo vs. BQ-123, vs. BQ-788 and vs. BQ-123/788, and BQ-123 vs. BQ-123/788.

On each study day, subjects underwent a standard clearance study. After baseline measurements, the low dose of antagonist was then administered followed by three 30 min collection periods. The higher dose of antagonist was then administered followed by 5 further 30 min collection periods. (Fig 5.1)

Fig 5.1 Study protocol



At 0, 60 & 90 min after the start of low and high dose antagonist, and at the end of the study, blood samples were taken for the measurement of plasma ET-1, aldosterone, ANG II concentrations and PRA. BQ-123 was measured before and at 15, 45 & 90 minutes after the start of each dose of antagonist, and at the end of the study.

5.2.1 Subjects

8 patients with stable CRF and 8 matched controls were recruited into the study. To enhance homogeneity and avoid other influences on vascular reactivity, patients with vasculitis, other systemic inflammatory disease, polycystic kidney disease, nephrotic syndrome or obstructive uropathy were excluded. Additionally, patients with significant co-morbid disease including diabetes mellitus, heart or lung disease, peripheral vascular disease or hypercholesterolaemia were excluded. The two study

groups were matched for age, weight, serum cholesterol and blood pressure (Table 5.1). Further patient characteristics are given in Table 5.2.

Table 5.1 Subject demographic data

	Healthy Volunteers	Renal Patients	*t*-test
Age (yr)	47 ± 5 (23 - 64)	46 ± 5 (25 - 67)	NS
Body mass index (kg/m^2)	26 ± 2 (18 - 31)	27 ±1 (24 – 33)	NS
MAP (mmHg)	92.8 ± 3.1 (83.0 – 103.6)	98.8 ± 3.5 (78.8 – 109.5)	NS
Creatinine (μmol/L)	85 ± 5 (62 – 111)	255 ± 41 (122 – 434)	p<0.01
Urinary Na excretion (mmol/24 hr)	136 ±14 (56 – 199)	150 ± 14 (74-246)	NS
Cholesterol (mmol/L)	5.3 ± 0.3 (3.9 – 6.1)	5.6 ± 0.2 (4.5 – 6.7)	NS
Urinary protein excretion (mg/24 hr)	0[†]	476 ± 110 (27 – 2033) (n=7)	p<0.01

[†]values below limit of laboratory assay

Table 5.2 Patient characteristics

Subject	Age (yr)	CrCl (ml/min)	Cause of renal impairment	Drugs	Other conditions
1	25	18	Renal calculi, single kidney	Enalapril, bicarbonate, 1-α calcidol	HT, Secondary ↑PTH
2	37	16	NK – late presentation	Lisinopril, bicarbonate, omeprazole, frusemide	HT
3	40	31	IgA nephropathy	Enalapril	HT
4	44	61	IgA nephropathy	Ranitidine	HT, PUD
5	50	36	Proliferative GN	Enalapril, metoprolol, nifedipine, allopurinol, frusemide	HT Gout
6	52	38	IgA nephropathy	Enalapril, doxazosin, bicarbonate, omeprazole	HT Gout
7	54	22	Proliferative GN	Fosinipril, atenolol	HT
8	67	59	IgA nephropathy/HSP	Labetalol	HT

CrCl - creatinine clearance, GN - glomerulonephritis, HSP - Henoch-Schonlein Purpura, HT - hypertension, NK - not known, ↑PTH - hyperparathyroidism, PUD - peptic ulcer disease, ↑PTH - hyperparathyroidism, UPE - urinary protein excretion

5.3 Results

Twelve renal patients were initially recruited: 1 developed nausea after receiving BQ-123, and 1 was unable to void urine at 30 min intervals. Two others withdrew for reasons unrelated to the study. Eight patients, and all healthy controls, completed the study without adverse events. Baseline study data are given in Table 5.3.

Table 5.3: Baseline data

	Healthy Volunteers	Renal Patients	*t*-test
MAP (mmHg)	94.0 ± 2.2 (86.5 – 104.9)	100.5 ± 4.0 (76.9 - 110.1)	NS
SVRI (dyne.sec/cm^5/m^2)	3089 ± 269 (1558 – 4068)	3479 ± 270 (2296 - 4478)	NS
CI (L/min/m^2)	2.6 ± 0.3 (1.9 – 4.6)	2.4 ± 0.2 (2.0 - 3.2)	NS
HR (bpm)	58.2 ± 2.3 (47.2 – 67.4)	56.6 ± 1.8 (49.1 - 62.4)	NS
ERBF (ml/min)	683 ± 41 (534 - 844)	295 ± 58 (81 - 571)	p<0.01
ERVR (mmHg.min/L)	148 ± 12 (108 - 191)	489 ± 101 (208 - 973)	p<0.01
EFF (%)	25.4 ± 2.0 (17.8 - 36.3)	20.8 ± 1.8 (13.5 - 30.7)	NS
GFR (ml/min/1.73m^2)	116 ± 11 (80 - 164)	39 ± 6 (19 - 67)	p<0.01
FWC (ml/min)	5.0 ± 0.5 (3.0- 7.5)	3.1 ± 0.5 (1.4 - 6.0)	P<0.05
UNaV (μmol/min)	143 ± 11 (56 - 215)	180 ± 31 (70 - 341)	NS
Urinary protein (mg/L)	0[†]	453 ± 82 (101 - 1435)[§]	p<0.01
Plasma ET-1 (pg/ml)	4.2 ± 0.3 (2.9 – 5.3)	5.6 ± 0.3 (4.6 - 7.3)	p<0.01
PRA (pg/ml/hr)	3.6 ± 0.5 (2.2 – 6.4)	10.5 ± 2.3 (3.3 - 20.6)	p<0.05
Plasma ANG II (pg/ml)	7.7 ± 1.0 (4.4 – 12.6)	7.6 ± 1.1 (4.2 – 13.8)	NS
Plasma aldosterone (pg/ml)	61 ± 4 (45 - 81)	88 ± 28 (33 - 274)	NS

*Values are given as the mean of the two baseline periods over the four study days ± SEM with the range of values given in brackets. NS = not significant [†]values below limit of laboratory assay, [§]n= 7 one value below limit of detection.

Fluid balance was calculated for each subject during each phase of the study. Not accounting for insensible losses, subjects were in a small positive fluid balance by the end of the 7.5 hr study (+51±21ml/hr for healthy subjects, +69±26 ml/hr for renal patients, p=0.58 healthy subjects vs. CRF). This did not differ with different phases of the study (placebo, BQ-123, BQ-123/788 or BQ-788) and was not different between the healthy subjects and patients with renal failure.

5.3.1 Systemic haemodynamics (Fig 5.2)

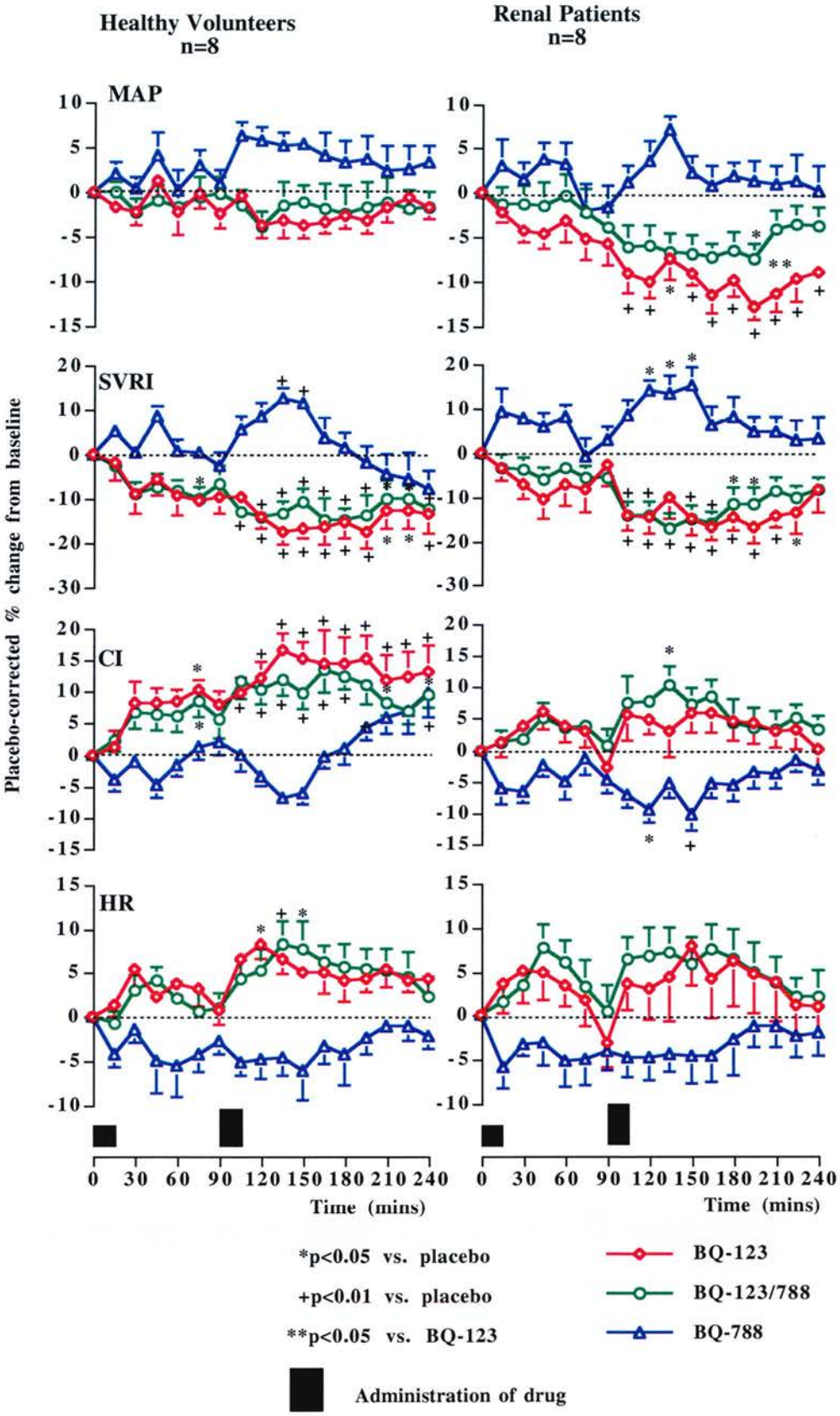
In CRF patients, placebo was associated with increases in SVRI to study end (3478±240 dyne.s m^2/cm^5 vs. 3797±301 dyne.s m^2/cm^5, $p<0.05$) and MAP (100.7±3.8 mmHg vs. 108.0±4.1 mmHg, $p<0.01$), consistent with the waning effects of anti-hypertensive medication. BQ-123 and BQ-123/788 reduced SVRI to a similar extent (BQ-123 (maximum placebo corrected change from baseline) –630±145 dyne.s m^2/cm^5, BQ-123/788: -617±158 dyne.s m^2/cm^5, both $p<0.01$ vs. placebo). MAP was reduced in CRF patients after BQ-123/788 (-7.4±1.6 mmHg, $p<0.01$ vs. placebo), but to a greater extent after BQ-123 alone (BQ-123: –12.9±1.7 mmHg, $p<0.01$ vs. placebo and BQ-123/788). The systemic haemodynamic response observed in individual patients was not related to baseline blood pressure.

In healthy controls, placebo did not alter systemic haemodynamics. Both BQ-123 and BQ-123/788 reduced SVRI to a similar extent, and equivalent to that seen in CRF (BQ-123: –591±104 dyne.s m^2/cm^5, BQ-123/788: -498±159 dyne.s m^2/cm^5, both $p<0.01$ vs. placebo). The reductions in MAP after BQ-123 & BQ-123/788 were equal (BQ-123: –3.6±1.4 mmHg, BQ-123/788: -3.7±1.6 mmHg, both $p<0.01$ vs. placebo), and less than those seen in CRF ($p<0.05$).

BQ-788 alone increased MAP (CRF: +7.0±1.6 mmHg, HC: +5.8±1.5 mmHg, both $p<0.01$ vs. placebo) and SVRI (CRF: +454±114 dyne.s m^2/cm^5, $p<0.01$ vs. placebo, healthy controls: +390±76 dyne.s m^2/cm^5, $p<0.05$ vs. placebo) to a similar extent in CRF patients and controls.

Increases in HR and CI were observed with selective ETA and combined ETA/B receptor antagonism, while decreases in these indices were observed following ETB receptor antagonism. While HR responses were similar between healthy volunteers and patients, the increases in CI with the dilator drugs were less, particularly for ETA receptor antagonism, and the reductions in CI following ETB receptor antagonism greater in patients compared to healthy volunteers.

Figure 5.2 Systemic haemodynamics after administration of ET receptor antagonists



5.3.2 Renal haemodynamics (Fig 5.3)

In CRF patients, BQ-123, but not BQ-123/788, produced striking increases in ERBF, and reductions in ERVR and EFF (ERBF: +102±74 ml/min, ERVR: -243±91 mmHg.min/L, EFF: -4.2±2.9%, all $p<0.01$ vs. placebo and vs. BQ-123/788). GFR did not change. By contrast, in healthy controls, BQ-123 and BQ-123/788 were neutral in respect of ERBF, ERVR, EFF and GFR.

In both groups, BQ-788 reduced ERBF (CRF -77±72 ml/min, HC –134±47 ml/min; both $p<0.05$ vs. placebo) and increased ERVR (CRF 112±63 mmHg.min/L, HC 39±12 mmHg.min/L; both $p<0.05$ vs. placebo). These changes were apparent even at low dose, and associated with a reduction in GFR and an increase in EFF.

5.3.3 Urinary free water clearance and sodium excretion (Fig 5.4)

Free water clearance was modestly increased by BQ-123/788 ($p<0.05$ vs. placebo) in healthy controls. A similar trend was seen after BQ-123 in healthy controls and after BQ-123 and BQ-123/788 in CRF, particularly after administration of the low dose of ET receptor antagonist, though this did not reach statistical significance. FWC was, however, reduced after BQ-788 administration in 7 out of 8 CRF patients. No changes in sodium excretion were observed in either CRF or healthy controls.

5.3.4 Urinary protein excretion (Fig 5.4)

Urinary protein excretion was undetectable for one CRF subject and all healthy controls. In the remaining 7 CRF patients, after correcting for GFR, BQ-788 and BQ-123/788 did not affect proteinuria, but BQ-123 reduced protein leak by 46% (–8.1±4.9 μg/ml) at 210 min ($p<0.01$ vs. placebo), an effect most apparent in subjects with higher baseline urinary protein excretion.

Figure 5.3 Renal haemodynamics after administration of ET receptor antagonists

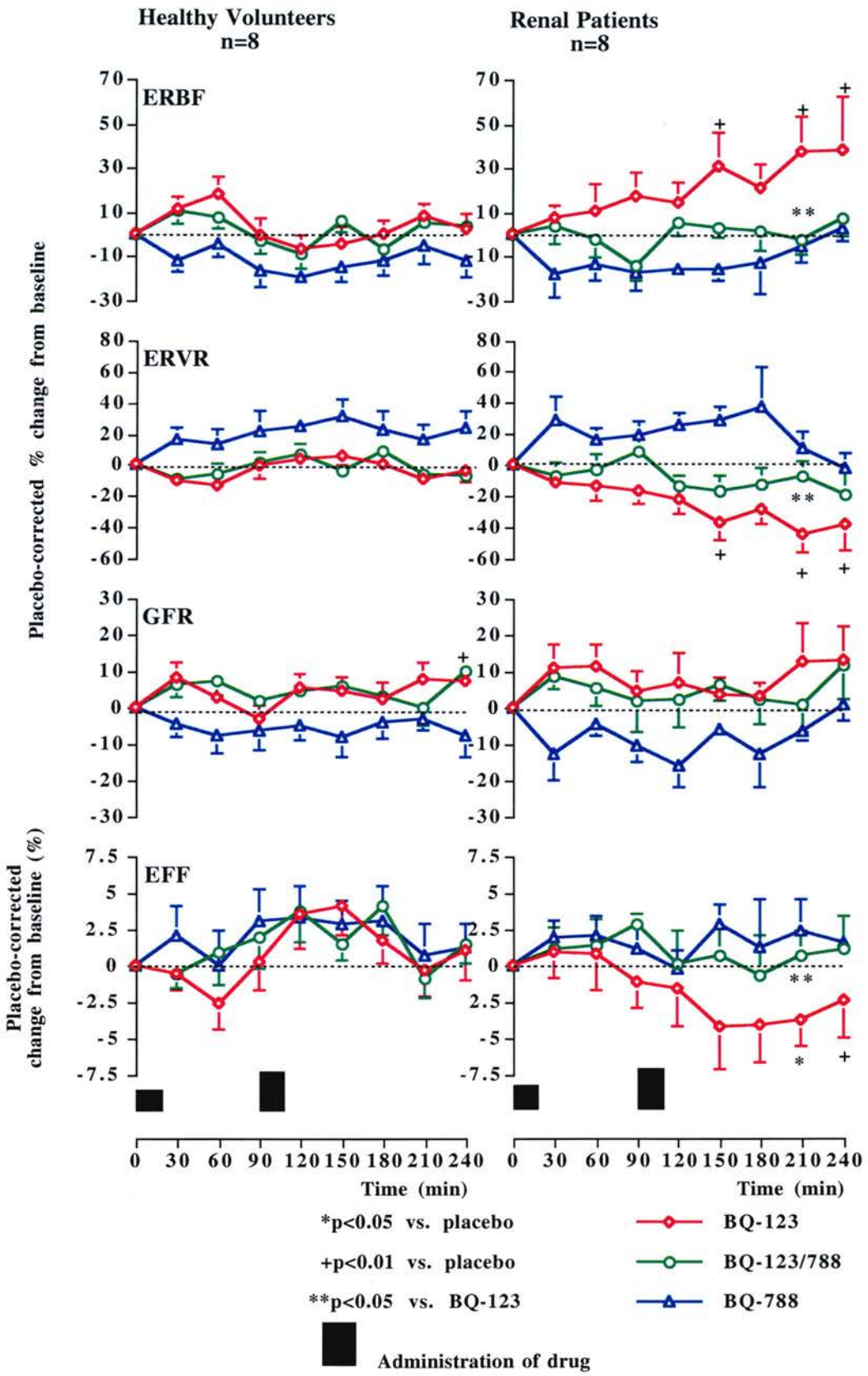
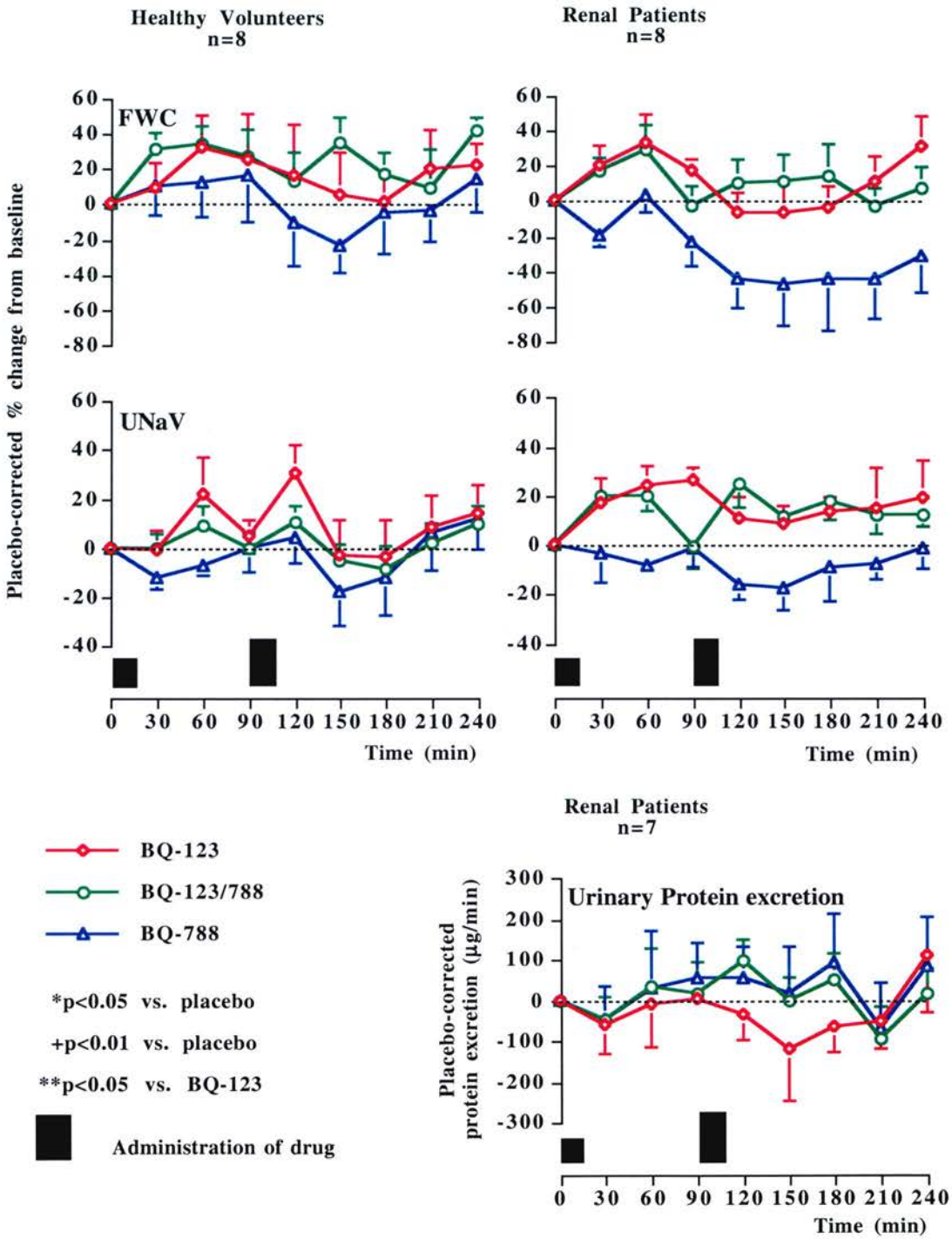


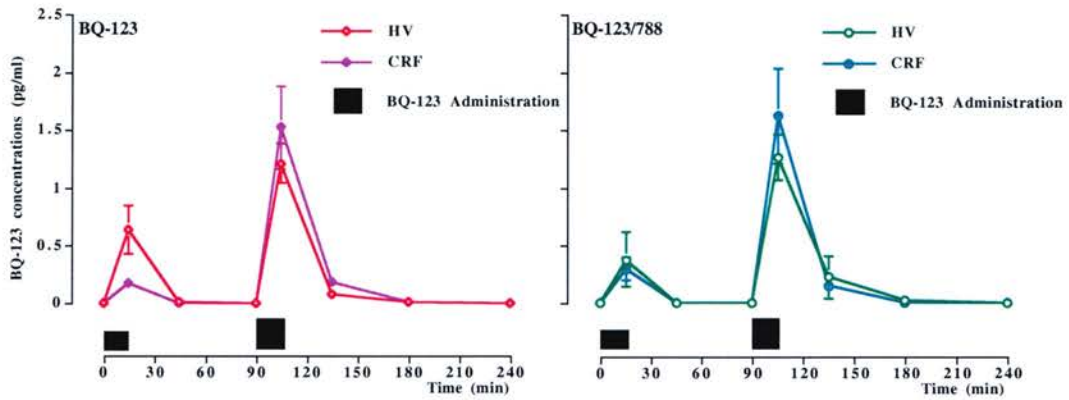
Figure 5.4 Free water clearance, urinary sodium excretion and protein excretion after administration of ET receptor antagonists



5.3.5 Plasma BQ-123 (Fig 5.5)

BQ-123 was detectable in plasma at 15 min post low dose and at 15 & 45 min post high dose administration. There was no difference in the plasma concentrations of BQ-123 between healthy controls and renal patients (CRF 1.52±0.36 pg/ml, HC 1.21±0.17 pg/ml at 15 min post high dose), nor between study days when BQ-123 was given alone compared with when given with BQ-788 (CRF 1.62±0.20 pg/ml, HC 1.26±0.20 pg/ml).

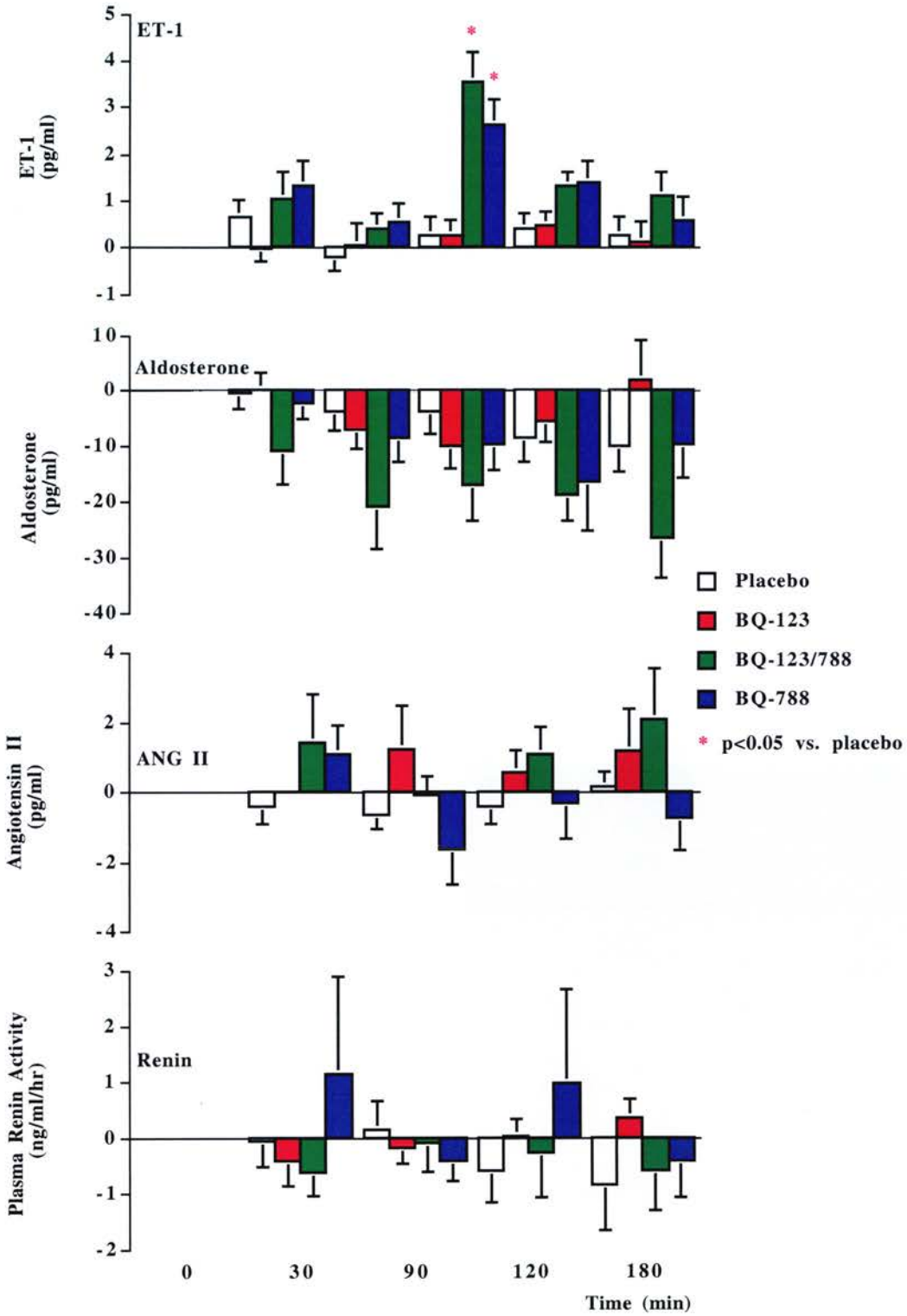
Figure 5.5 Plasma BQ-123 concentrations after BQ-123 administration ± BQ-788



5.3.6 Plasma hormone concentrations (Fig 5.6)

Baseline ET-1 concentrations were slightly higher in renal patients compared to healthy volunteers (Table 5.3). Plasma ET-1 concentrations were increased o a similar extent after high dose BQ-788 (Healthy controls baseline: 4.1 ± 0.4 pg/ml, 120 min: 6.6 ± 1.0 pg/ml, CRF baseline: 5.6 ± 0.4 pg/ml, 120 min: 8.3 ± 0.5 pg/ml) and BQ-123/788 (Healthy controls baseline: 3.9 ± 0.5 pg/ml, 120 min: 8.5 ± 1.1 pg/ml, CRF baseline: 5.4 ± 0.3 pg/ml, 120 min: 7.9 ± 0.4 pg/ml), but unaltered by placebo or BQ-123. Plasma renin activity, aldosterone and ANG II concentrations were unchanged by the administration of placebo, BQ-123 and/or BQ-788.

Figure 5.6 Plasma hormone concentrations after ET receptor antagonists



5.4 Discussion

This is the first clinical study to directly compare ETA, ETB and combined ETA & ETB receptor antagonism at systemic doses in man. It demonstrates that, in CRF patients, that selective ETA receptor antagonism produces substantial reductions in blood pressure that are associated with renal vasodilatation, without changes in GFR. Combined ETA/B receptor blockade was less effective in lowering blood pressure, had no effect on renal haemodynamics and reduced ET-1 clearance. Selective ETB receptor antagonism alone produced substantial systemic and renal vasoconstriction. By contrast, in healthy subjects the effects of ETA and ETA/B receptor blockade on blood pressure were similar to each other, but less than those seen in CRF, and there were no effects on renal haemodynamics.

These data confirm the physiological importance of ET- 1, through activation of the ETA receptor, in the maintenance of basal systemic but not renal vascular resistance [140]. They also show that ET-1 plays a major role in regulating blood pressure and renal vascular resistance in CRF, consistent with activation of the ET system in this condition. Additionally, the reduction in EFF suggests an effect primarily on efferent arteriolar tone, which may serve to reduce glomerular pressure, and thus be renoprotective.

The effects of BQ-788, whether in the presence of BQ-123 or not, suggest the net effect of ETB receptor activation on the circulation, in health and renal disease, is to produce vasodilatation. Therefore, similar to observations in healthy subjects [194, 210] and patients with heart failure [388], any enhanced effects of constrictor ETB receptors in CRF is outweighed by ETB receptor mediated vasodilatation. Of interest, ETB receptor antagonism increased renal vascular resistance twice as much (~20-30%) as systemic vascular resistance (~10-15%), suggesting that tonic ETB receptor mediated renal vasodilatation plays a key role in maintenance of renal vascular tone. This is likely to be of particular importance in CRF, where baseline renal vascular resistance is high.

This study has demonstrated a reduction in SVRI after BQ-123 or BQ-123/788 that is similar in patients and healthy volunteers. Using a nitric oxide clamp, Verhaar *et al* demonstrated that the vasodilatory response of the forearm circulation to ETA receptor antagonism was dependent on NO production [194]. As many cardiovascular disease states including CRF are characterised by a reduction in NO activity [389-391], this finding would suggest that ETA receptor blockade should be less effective in disease. Consistent with this, forearm studies in renal failure patients [195], heart failure patients [388], and hypertensives [222] have demonstrated a reduced vasodilatory response to BQ-123 when compared to healthy controls. This was clearly not the case in this study.

BQ-123 concentrations were the same in the CRF patients and the healthy controls, suggesting that altered pharmacokinetics does not account for this difference. Indeed as BQ-123 is metabolised by the liver [97] one would not expect altered pharmacokinetics in renal patients (see Chapter 11 for further exploration of BQ-123 pharmacokinetics). It is possible that the medications taken by renal patients may be responsible for this finding. As the duration of the study was a minimum of four weeks plus one week washout period for each individual, withdrawal of medications was felt not only to be ethically unjustified, but it also introduced another unmatched variable, hypertension, compared to the healthy controls that might have affected the response to ET antagonists. Given the prevalence of hypertension in renal disease, it was felt that the option chosen (to maintain patients on their medication) was a clinically relevant one and in keeping with previous studies in heart failure patients [293, 294, 299]. In this respect, it is important to note that six of the eight patients were taking ACE inhibitors. These drugs, in addition to blocking the conversion of ANG I to ANG II, also increase NO activity via inhibition of bradykinin breakdown [392]. It is possible therefore that ACE inhibitors and ETA antagonists, both acting through NO, somehow interact to increase the haemodynamic response observed. An acute synergism between ETA receptor antagonism and ACE inhibitors has been demonstrated in animals in respect of systemic and renal haemodynamics [393] but has yet to be studied in man. In Chapter 6 and 7, this issue is addressed in healthy volunteers.

However, despite similar changes in SVRI, blood pressure fell significantly in renal patients, while healthy volunteers defended their blood pressure. Reductions in MAP in the healthy volunteers are small and similar to those seen in Chapter 3 and in other studies [140, 200, 202]. Our experience is that healthy subjects defend their blood pressure via reflex alterations in HR and CI, and that MAP is usually the last cardiovascular measurement to change, an observation supported by this study. It is potentially one reason why studies with ET receptor antagonists in healthy volunteers that only measure mean arterial pressure, and do not look at vascular resistance, have failed to show significant effects [139, 202]. In the renal patients, the larger reduction in blood pressure is accompanied by a lesser increase in CI in patients compared healthy volunteers, suggesting that the hypertensive renal heart less able to compensate for changes in SVRI. The greater reduction in MAP in patients after ETA receptor antagonists and corresponds with a smaller increase in CI with ETA compared to ETA/B receptor antagonism. It is interesting to speculate whether this is a direct cardiac effect of ETA receptor blockade, though this study was not designed to answer this question.

Consistent with previous studies [120, 139-141, 199], selective ETA and non-selective ETA/B receptor antagonists had little effect on ERBF or ERVR in healthy subjects. As the higher dose of BQ-123 is at the top of the dose response curve for systemic haemodynamics (Chapter 3) insufficient dose is an unlikely explanation for the lack of effect of ETA and non-selective antagonism on the renal circulation of healthy volunteers. It is possible that the ETA receptor is not an important mediator of renal vasoconstriction in health. However, the circumstances of this study mean that this is an unchallenged renal circulation and therefore likely to be maximally dilated. It is possible therefore that no effect is seen as no further dilatation can occur.

In patients however, ETA blockade produces a clear reduction in ERVR and increase in ERBF. The increased ERVR at baseline compared to healthy controls is a good indicator that, in the renal circulation in patients, even unchallenged, the balance

between constrictors and dilators is shifted towards the constrictors. Blockade of one of these constrictor systems i.e. ET-1 will, therefore, produce a dilatation. The response in renal patients to selective ETA receptor would suggest that the ETA receptor mediates ET-1 induced renal vasoconstriction. The neutral effect of non-selective receptor antagonism on ERBF and ERVR underlines the importance of the vasodilatory action of the ETB receptor in the renal circulation of CRF patients. Loss of this vasodilatory action, in contrast to the systemic circulation, reduces the effectiveness of ETA receptor blockade. Again this study provides no evidence to support an upregulation of vasoconstrictor ETB receptors in the renal circulation.

The renal vasoconstriction produced by selective ETB receptor antagonism is accompanied, not surprisingly, by a reduction in GFR. EFF is moderately increased, suggesting, in the absence of changes in filtration coefficient, an increase in efferent arteriolar tone. The reduction in EFF observed with ETA receptor antagonists in renal patients is also suggestive of a preferential efferent arteriolar effect. If this is real, it implies that the observed increase in RBF is not associated with increases in glomerular capillary perfusion. This is important as an increase in ERBF accompanied by increases in glomerular capillary perfusion pressure could be deleterious to long term renal function, conversely a fall in glomerular capillary perfusion pressure would result in a situation analogous to ACE inhibitors, and might be expected to improve long term renal outcome. Consistent with our observations, an ETA receptor selective antagonist has been shown to reduce proteinuria in type 1 diabetics [190]. However, it would mean that ETA receptor blockade would need to be approached with caution in situations of low renal perfusion pressure e.g. congestive heart failure and renovascular disease. Because of the importance of this issue to rational prescribing of ET receptor antagonists, Larger, chronic studies are needed to confirm or refute this finding.

Perhaps surprisingly, given the evidence for ETB receptor mediated natriuresis shown in animal studies [394], no changes in sodium excretion were observed in this study. However, the urinary sodium data in this study were the most variable and failed to achieve significance. Larger studies are required to adequately define the

renal tubular actions of ET receptor antagonists. Despite this, during ETA receptor blockade, in the face of substantial systemic and renal vasodilatation, sodium retention did not occur, which is important if these drugs are to be safely prescribed to patients with CRF.

Based on the data from study 1 and earlier work, this study was designed to achieve effective and selective blockade of the ETA and ETB receptor ([194, 210, 395] and Chapter 3) and not specifically to reproduce the effects of existing ET receptor antagonists, which all block ETA to a greater extent than ETB receptors [396]. Therefore, although the main effects of therapeutic interest in our study appear to derive from ETA receptor blockade, and are countered by ETB blockade, drugs causing only modest ETB receptor inhibition might produce similar effects. As a limitation, ours were acute studies. However, based on studies in heart failure, [293] haemodynamic effects would be sustained, or even greater, with continued treatment. Also, a relatively homogeneous CRF population were recruited to this study. Therefore, further work is now needed in a broader population of patients with kidney disease, including those with low renal perfusion pressure. In particular, as ACE inhibitors are most effective as renoprotective agents in patients with >3g/24hr of proteinuria, studies are needed in patients with higher degrees of urinary protein leak to address what may be a major benefit of these agents.

In conclusion, this study suggests that selective ETA receptor antagonists may be valuable anti-hypertensive drugs in patients with CRF as well as offering additional benefits, including renoprotection. ET receptor antagonists also improve endothelial function [397], reduce inflammation and fibrosis [398], and reverse cardiac [399] and vascular remodelling [400] and so may offer additional benefits to renal patients, who are at high cardiovascular risk. On this basis, longer term studies in patients with CRF are justified, paying particular attention to effects on proteinuria as a surrogate marker for the progression of renal disease.

Chapter 6

An examination of the interaction between ACE inhibition and ET receptor blockade in healthy human volunteers

6.1 Introduction

ET-1 and ANG II are both powerful vasoconstrictors involved in the regulation of vascular tone, and there is considerable evidence for an interaction between the ET and renin-angiotensin-aldosterone systems [401]. ANG II increases ET-1 transcription and secretion *in vitro* in a variety of cell types, including endothelial and vascular smooth muscle cells [21, 402], and ET-1 can increase ACE activity [73, 403]. As discussed in Chapter 4, ET receptor antagonists attenuate the acute haemodynamic effects of ANG II in rats *in vivo* [377, 379], although this is by no means a uniform finding [375], and has not been replicated in acute studies in dogs [376] or in local [404] or systemic studies in man (Chapter 4). Animal data have, however, demonstrated that ACE inhibition can attenuate ET-1 induced hypertension [405, 406] and suggested that concomitant blockade of the renin-angiotensin and ET systems produces changes greater than those seen with blockade of either system alone [393, 407-409]. In man, synergism between ETA receptor antagonists and angiotensin receptor type 1 antagonists has been demonstrated [410]. Also, many clinical studies with ET receptor antagonists demonstrating clear haemodynamic effects [293, 299] have been performed in patients already receiving ACE inhibitors.

In contrast to forearm studies that have suggested that ETA receptor blockade would be less effective in patient groups compared to healthy controls [195, 222, 388], study 3 (Chapter 5) demonstrated that, despite a greater reduction in blood pressure in CRF patients, reductions in SVRI were similar in CRF patients and healthy volunteers. In light of the above evidence, a possible explanation for the augmented response in CRF, as six of the eight patients were or ACE inhibitors, might be due to an interaction between ET receptor antagonism and ACE inhibition.

The aim of this study was, therefore, to explore the possible systemic and renal interaction between ET receptor antagonism and ACE inhibition, hypothesising that pre-treatment with ACE inhibition would augment the systemic and renal haemodynamic response to ETA receptor antagonism.

6.2 Study design

This was a randomised, double-blind, placebo-controlled study in 6 healthy subjects. For demographic data, see Table 6.1. Subjects attended for 6 visits, each separated by ≥7 days. On visits 1-3, they received either placebo, BQ-123, or the combination of BQ-123 & BQ-788 in a randomised order. They then followed and identical study protocol on visits 4-6, after pre-treatment with enalapril (E). The response to BQ-123 and BQ-123/788 in the presence or absence of E was pre-identified as the comparison of interest.

For 5 days before each study, subjects took enalapril at a dose of 20 mg twice daily (see methods for justification of dosing schedule) taking the last dose at 0830 on the study day. Subjects then underwent a standard clearance study. After baseline measurements, the low dose of antagonist was then administered followed by three 30 min collection periods. The higher dose of antagonist was then administered followed by 5 further 30 min collection periods.

Table 6.1 Subject demographic data

Age (yr)	47 ± 5 (23 - 64)
Body mass index (kg/m^2)	25 ± 2 (18 - 31)
MAP (mmHg)	88.5 ± 2.7 (71.4 – 99.4)
Creatinine (μmol/L)	89 ± 7 (62 – 119)
Urinary Na excretion (mmol/24 hr)	118 ± 15 (64 – 185)
Cholesterol (mmol/L)	5.2 ± 0.3 (3.9 – 6.1)

At 0, 60 & 90 min after the start of low and high dose antagonist, and at the end of the study, blood samples were taken for the measurement of plasma ET-1, aldosterone, ANG II concentrations and PRA. BQ-123 was measured before and at 15, 45 & 90 minutes after the start of each dose of antagonist, and at the end of the

study and serum ACE activity was determined at 0830 am and at 1430 (60 min after the start of the higher dose antagonist).

6.3 Results

All subjects completed all phases of the study without adverse events. Baseline study data (Table 6.2) were not different for any of the 6 individual phases of the study except for plasma aldosterone concentrations which were reduced during the phases when BQ-788 was administered by pre-treatment with enalapril.

Table 6.2: Baseline data

	Placebo	BQ-123	BQ-123/ 788	Enalapril+ Placebo	E+BQ-123	E+BQ-123 /788	No E vs. E *t-test*
MAP (mmHg)	96.2±2.5	92.6±4.1	94.0±3.2	87.2±1.7	89.2±3.8	90.6±5.0	p<0.01
SVRI (dyne.s m^2/cm^5)	2868±318	3157±435	3157±410	2726±305	2831±358	2616±353	p<0.01
CI ($L/min/m^2$)	2.94±0.47	2.64±0.43	2.60±0.38	2.77±0.39	2.73±0.36	2.99±0.36	NS
HR (bpm)	61.2±1.5	60.8±1.5	61.3±2.2	60.3±2.5	59.1±3.6	61.8±2.0	NS
ERBF (ml/min)	587±53	705±66	703±84	795±89	723±138	945±101	p<0.05
ERVR (mmHg.min/L)	173±19	139±15	142±21	119±16	142±22	108±18	p<0.05
EFF (%)	28±4	23±2	23±3	21±2	24±3	19±2	NS
GFR ($ml/min/1.73m^2$)	106±9	110±14	103±13	106±7	103±6	107±9	NS
UNaV (µmol/min)	157±19	150±32	134±29	154±13	142±22	157±28	NS
Plasma ET-1 (pg/ml)	4.7±0.5	4.7±0.3	3.5±0.2	3.8±0.3	3.6±0.3	3.9±0.1	NS
Plasma Aldosterone (pg/ml)	61±14	53±8	76±12	42±4	43±4	29±3*	p<0.05

However comparing the 18 visits where subjects received pre-treatment with enalapril to those where they did not, MAP, SVRI, ERVR and aldosterone were significantly reduced and ERBF was significantly increased.

6.3.1 Systemic haemodynamics

Administration of placebo, either alone or in the presence of pre-treatment with enalapril, did not alter systemic haemodynamics. Both selective ETA receptor antagonism with BQ-123, and combined ETA/B receptor antagonism with BQ-123 & BQ-788, reduced MAP (maximum placebo corrected change from baseline: BQ-123: –4.2 ± 1.6 mmHg, ANOVA $p<0.05$ vs. placebo; BQ-123/788: -4.4 ± 2.0 mmHg). After pre-treatment with E, the effect of BQ-123 on blood pressure was almost doubled (–8.3 ± 3.0 mmHg, $p<0.01$ vs. BQ-123 alone). However, this synergy was not seen when BQ-788 was co-administered with BQ-123 (–4.4 ± 2.9 mmHg) (Fig 6.1). SVRI followed a similar pattern (Fig 6.2). Pre-treatment with E was associated with a trend towards a smaller increase in HR after BQ-123 or BQ-123/788. No significant changes in CI were noted.

6.3.2 Renal haemodynamics

Placebo, E alone, BQ-123 and BQ-123/788 were all neutral in respect of ERBF, ERVR, EFF and GFR. After pre-treatment with E, BQ-123 increased ERBF (+146 ± 41 ml/min, $p<0.01$ vs. placebo and vs. BQ-123 alone), reduced ERVR (-32 ± 15 mmHg.min/L, $p<0.01$ vs. placebo and vs. BQ-123 alone) and reduced EFF (-3.4 ± 2.5%, $p<0.05$ vs. placebo, $p<0.01$ vs. BQ-123 alone). By contrast, after pre-treatment with E, BQ-123/788 increased ERBF (-224 ± 31 ml/min, $p<0.01$ vs. placebo and vs. BQ-123/788 alone) and reduced ERVR (26 ± 6 mmHg.min/L, $p<0.05$ vs. BQ-123/788 alone) (Fig 6.3-6.).

Figure 6.1 MAP

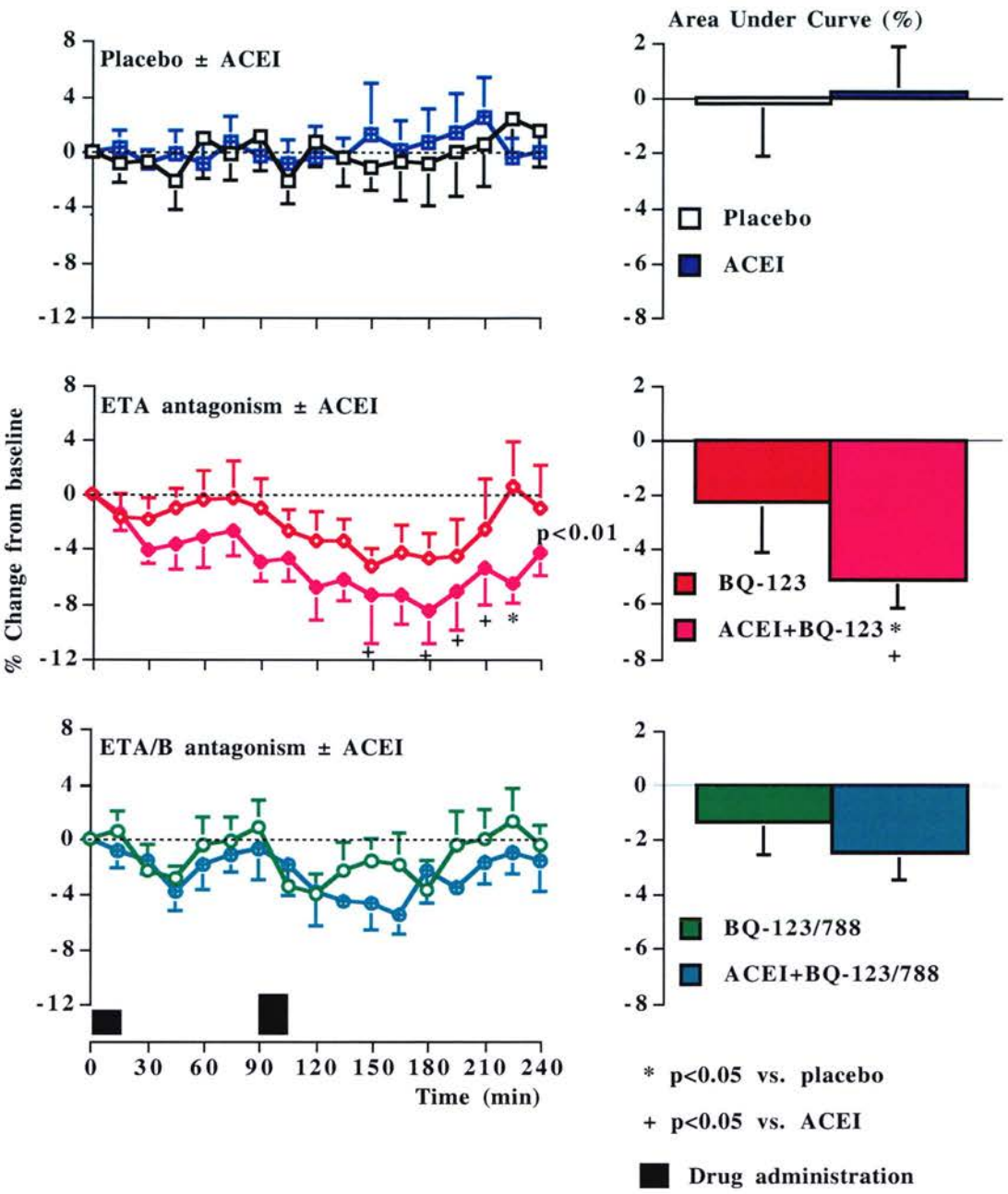


Figure 6.2 SVRI

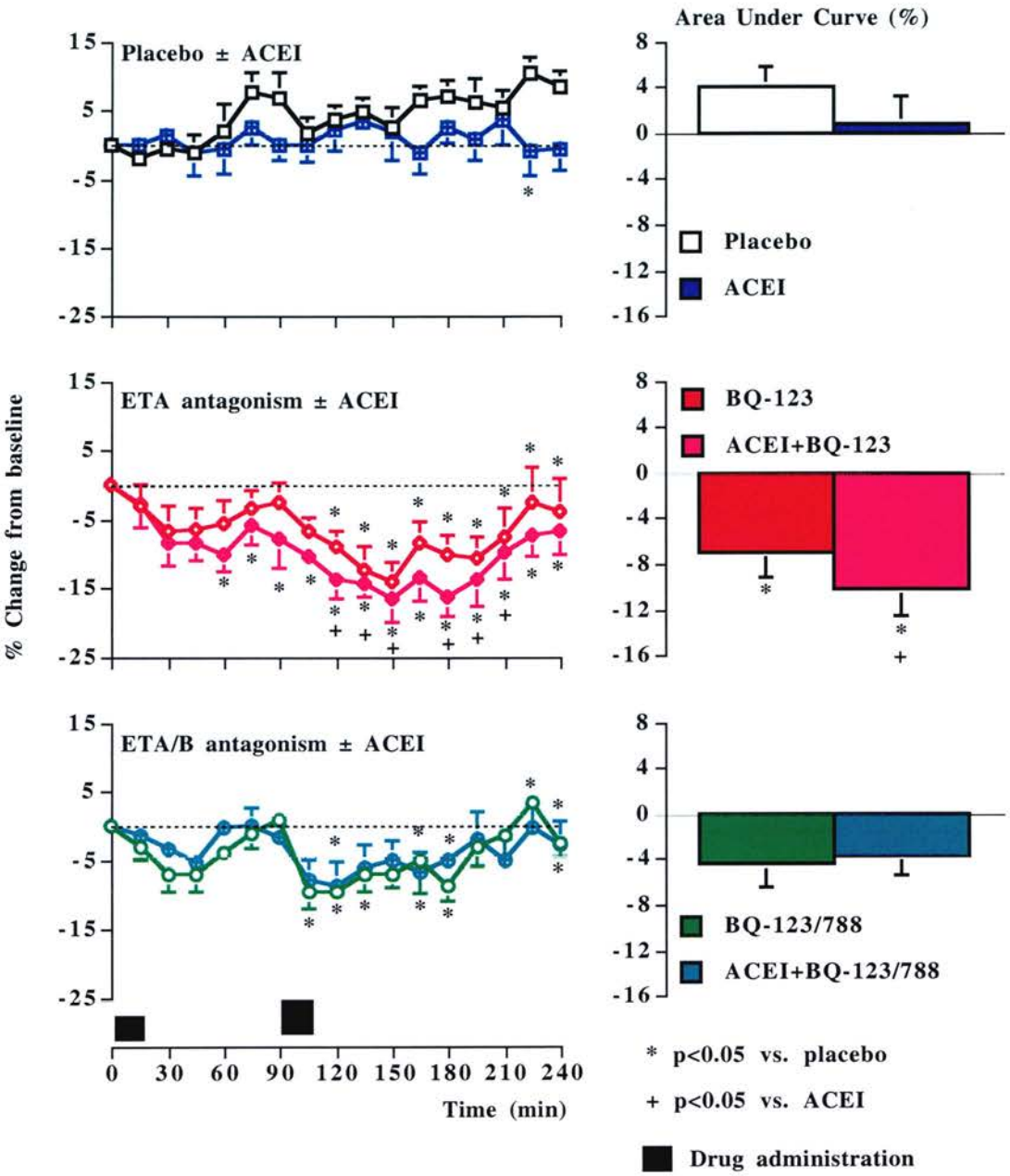


Figure 6.3 ERBF

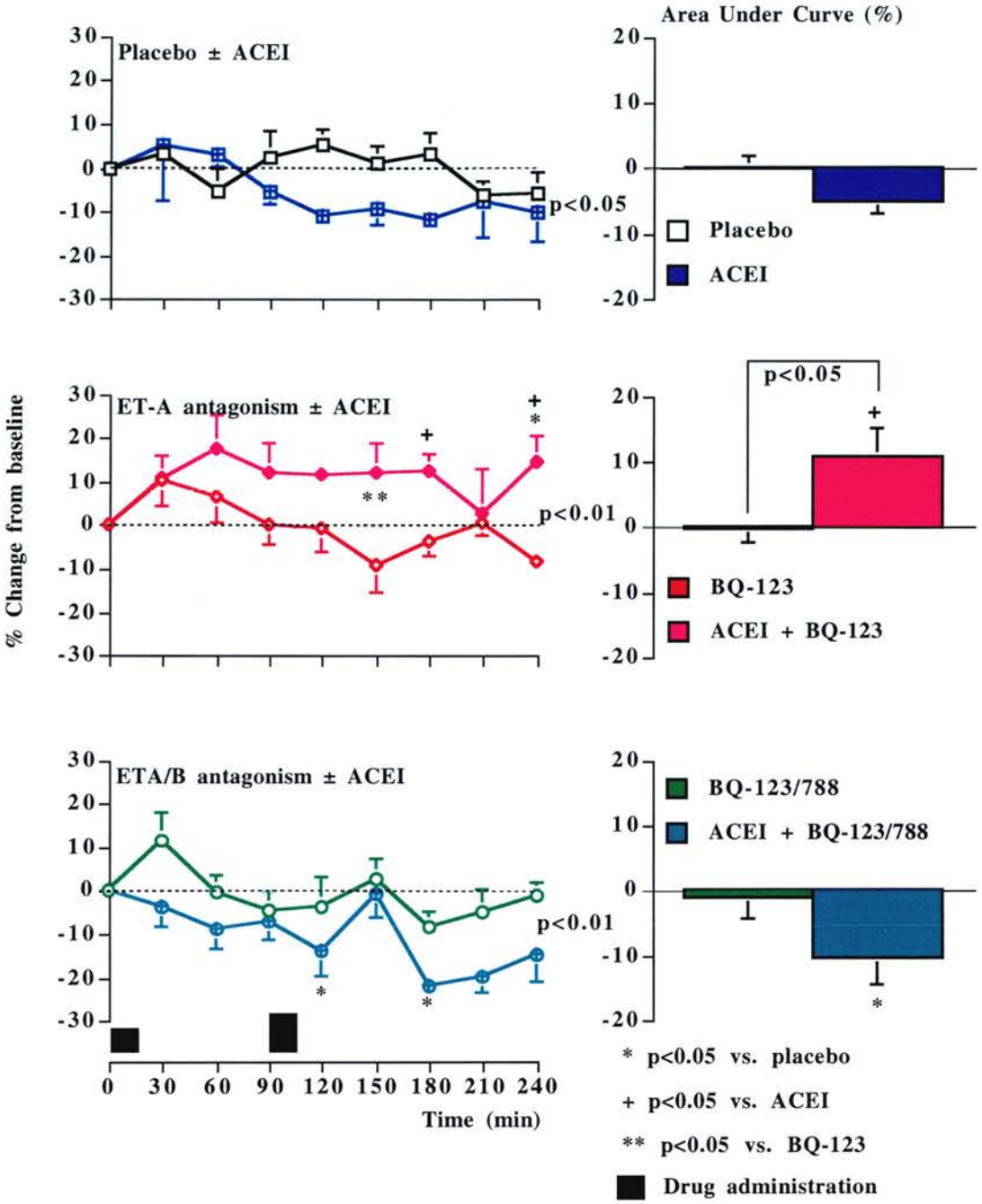


Figure 6.4 ERVR

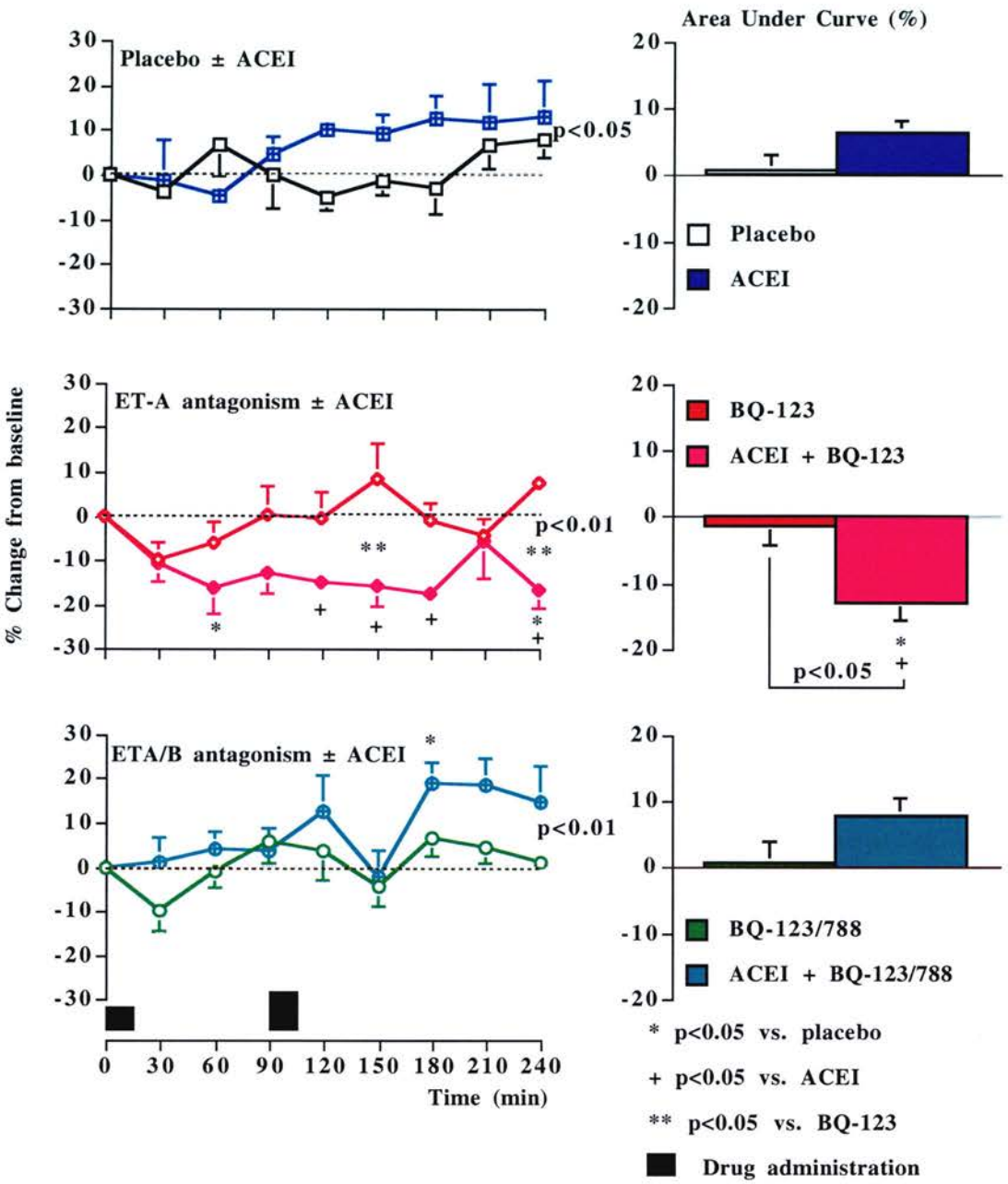


Figure 6.5 GFR

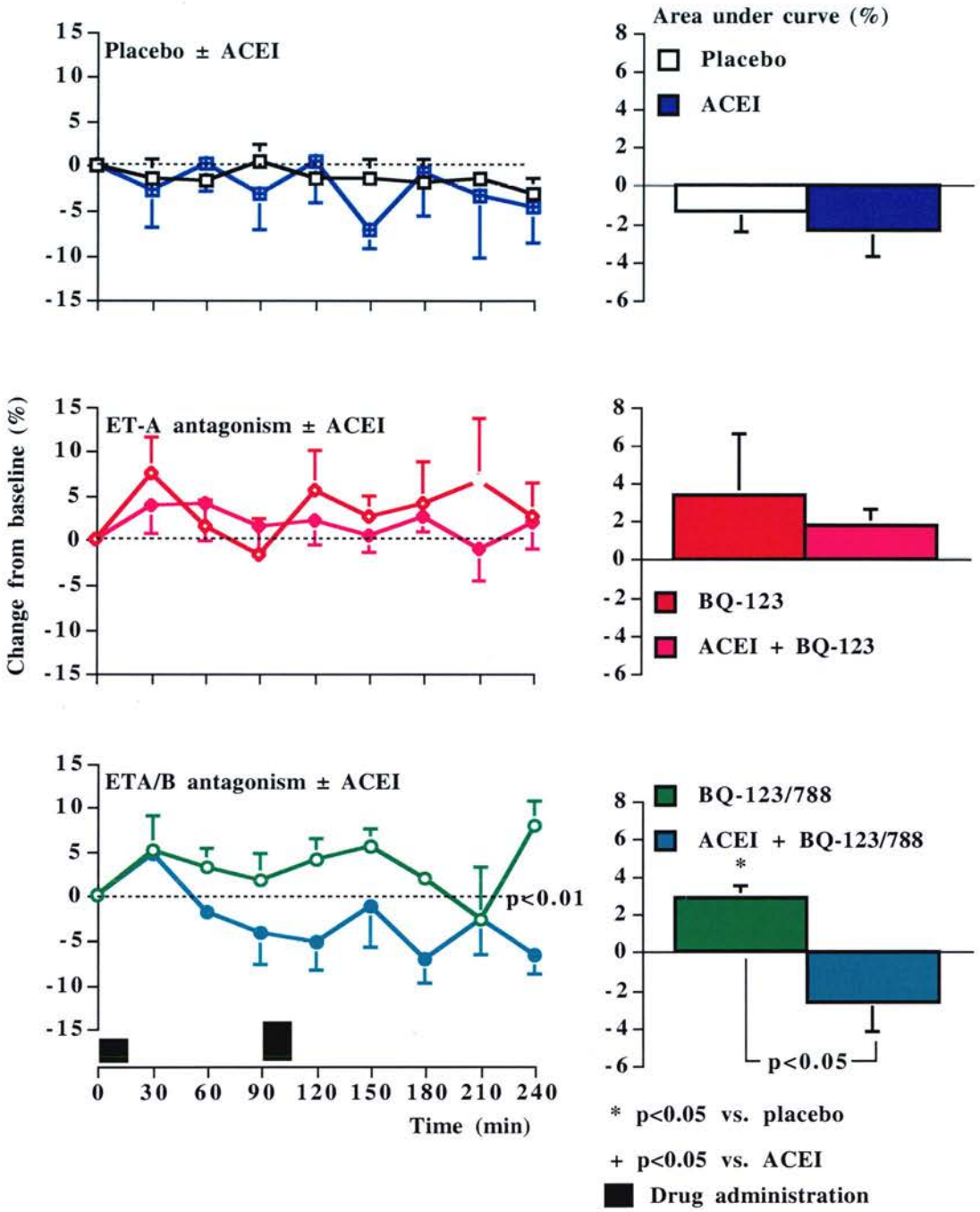
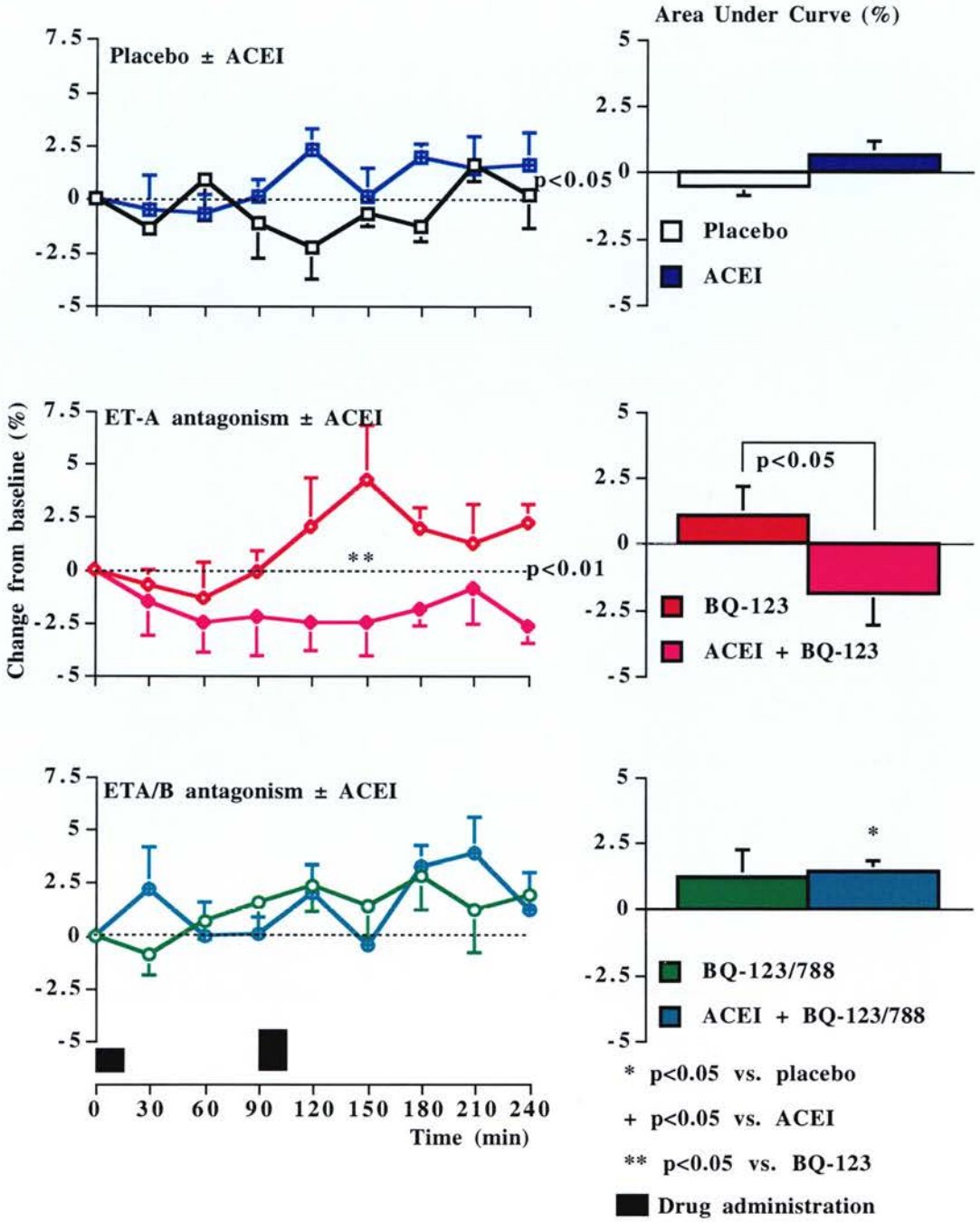


Figure 6.6 EFF



6.3.3 Urinary sodium excretion

No significant changes in natriuresis were observed after placebo, E alone and both BQ-123 or BQ-123/788 alone. However, after pre-treatment with E, BQ-123 produced a striking increase in urinary sodium excretion (UNaV: +58 ± 27 μmol/min, $p<0.01$ vs. placebo and BQ-123 alone). Fractional excretion of sodium followed a similar pattern. As with renal haemodynamics this increase was not observed with BQ-123/788. (Fig 6.7)

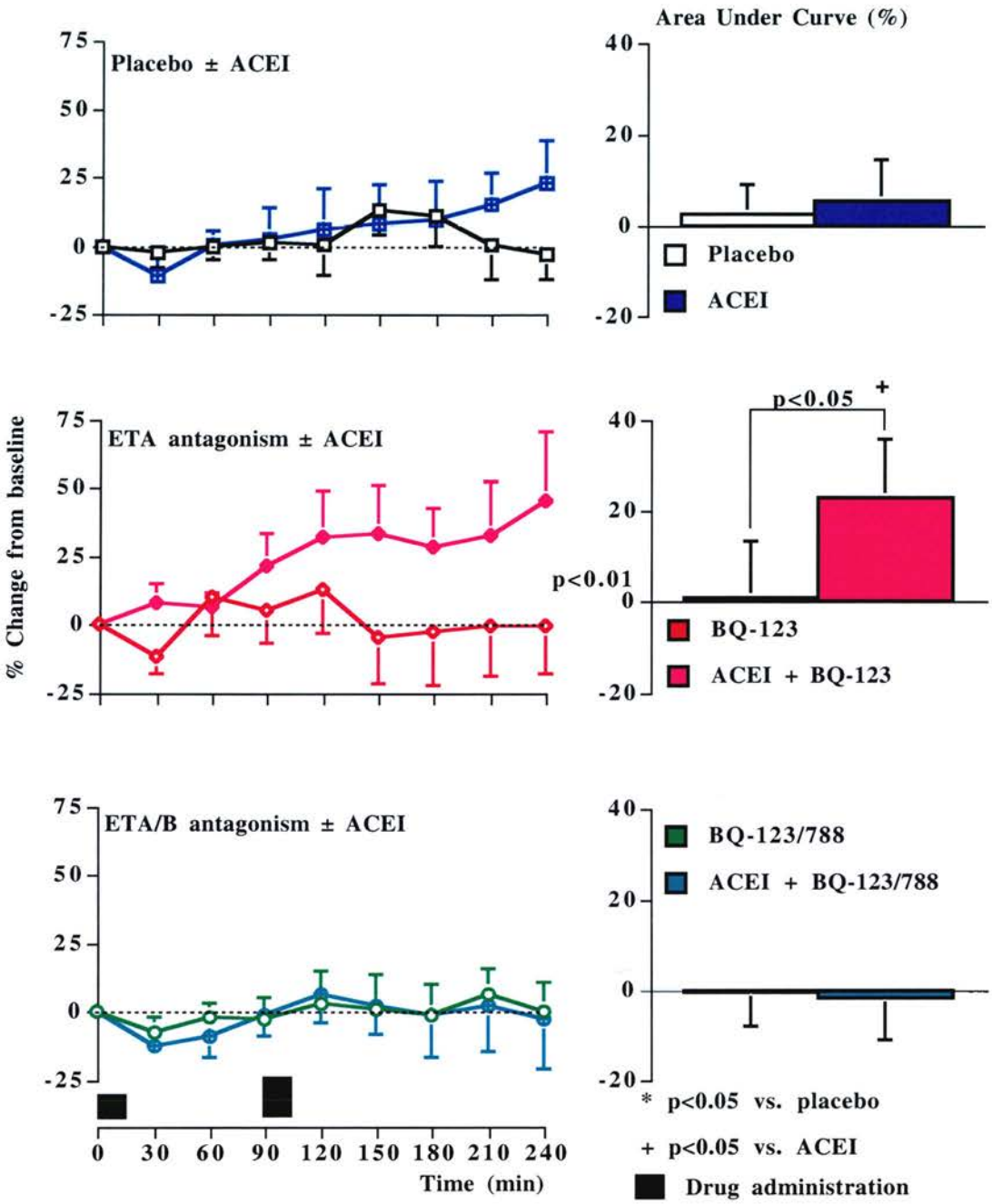
6.3.4 Serum ACE activity

Pre-treatment with E reduced serum ACE activity by 75% at baseline compared to no pre-treatment (9.1 ± 2.3 vs. 36.4 ± 4.3 U, $p<0.01$), and by 79% at 150 min (5.4 ± 1.7 vs. 26.5 ± 2.1 U, $p<0.01$). ET-receptor antagonist administration did not alter ACE activity (Table 6.3)

Table 6.3 Serum ACE activity after ET receptor antagonist administration

	Placebo	**BQ-123**	**BQ-123/788**
t=0 min	36.1 ± 3.2	39.0 ± 11.5	34.1 ± 6.5
t=210 min	27.4 ± 3.5	27.5 ± 2.7	24.6 ± 5.2
	Enalapril	**Enalapril +BQ-123**	**Enalapril +BQ-123/788**
t=0 min	10.6 ± 3.0	8.6 ± 3.0	11.7 ± 6.6
t=210 min	8.0 ± 3.2	3.7 ± 2.0	7.0 ± 3.9

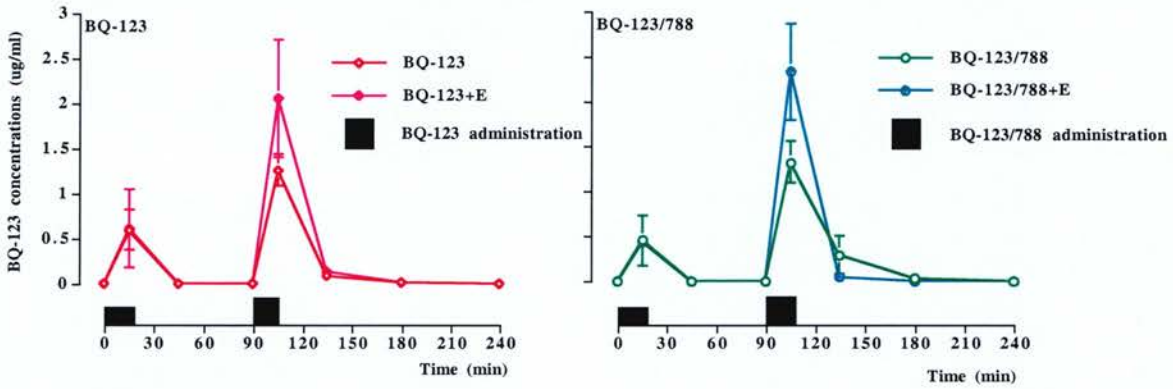
Figure 6.7 UNa V



6.3.5 Plasma BQ-123

BQ-123 was detectable in plasma at 15 min post low dose and at 15 & 45 min post high dose administration. Peak BQ-123 concentrations were higher after pre-treatment with E, though the difference was not statistically significant (BQ-123: 1.26 ± 0.18 pg/ml, BQ-123/788:1.32 ± 0.23, BQ-123 + E: 2.06 ± 0.65, BQ-123/788 + E: 2.32 ± 0.53) (Fig 6.8).

Figure 6.8 Plasma BQ-123 concentrations



6.3.6 Plasma ET-1 concentrations

Baseline plasma ET-1 concentrations were not altered by pre-treatment with enalapril (Table 6.2). They were increased following administration of BQ-788 whether administered in the presence of enalapril (+1.7 ± 0.6 pg/ml, 43 ± 16% $p<0.05$ vs. baseline) or not (4.4 ± 1.3 pg/ml, 132 ± 33%, $p<0.05$ vs. baseline), but unaffected by placebo or BQ-123 alone.

6.3.7 Plasma aldosterone concentrations

Baseline plasma aldosterone concentrations were lowered by pre-treatment with enalapril (pre treatment with enalapril 37.8±2.8 pg/ml, no pre-treatment 63.6±7.7 pg/ml, $p<0.05$) (Table 6.2). After administration of BQ-788, plasma aldosterone concentrations fell whether administered in the presence of enalapril (-10.6 ± 3.5 pg/ml, -38 ± 14% $p<0.05$ vs. baseline) or not (-33.3 ± 9.3 pg/ml, -40 ± 6%, $p<0.05$ vs. baseline), but unaffected by placebo or BQ-123 alone.

6.4 Discussion

This study demonstrates a synergy between ACE inhibition and ETA receptor antagonism, both on systemic and renal haemodynamics, and on renal tubular function that is lost when the ETB receptor is concomitantly blocked.

The renin-angiotensin and ET systems are known to interact [401] and an *in vivo* synergistic effect between ACE inhibition and ETA receptor antagonism has been demonstrated in animals [393], and in man between ERAs and angiotensin receptor antagonists [410]. This study has shown that, in healthy volunteers subjected to a high degree of ACE inhibition, the effects of ETA receptor blockade on systemic haemodynamics are enhanced. In respect of blood pressure, ACE inhibition almost doubles the effect of ETA receptor blockade. Additionally, in contrast to the absence of an effect of ETA receptor antagonism alone on the renal circulation, the combination with ACE inhibition increased renal perfusion, with an associated fall in EFF, and thus, by inference, a preferential vasodilatation of the efferent arteriole and a fall in glomerular capillary pressure. These alterations in glomerular haemodynamics have the potential to be renoprotective in a manner analogous to the effect of ACE inhibition alone. Additionally, a striking natriuresis was observed that was still developing at the end of the study, 4 hours after initial administration of BQ-123. If sustained over 24 hrs, this would be equivalent to 80 mmol of sodium excreted, a clinically important degree of sodium loss.

These results also demonstrated that, while maximal haemodynamic changes occurred after the higher dose of BQ-123, useful systemic and renal haemodynamic changes were achieved after the lower dose of BQ-123 in the presence of ACE inhibition. As adverse effects in clinical trials with ET receptor antagonists appear to be largely dose related [295], this combination might allow the use of a lower dose of ETA receptor antagonist without losing efficacy.

Montanari and colleagues [410] have demonstrated that ETA receptor antagonism can produce renal haemodynamic changes under conditions of ANG II receptor-1 blockade. This study demonstrates a similar synergy between ETA receptor

antagonism and ACE inhibition. Recently, enalapril has been shown to attenuate ET-1 induced hypertension (but not the reduction in GFR) in rats via increased bradykinin [406]. However, the loss of this synergistic effect in this study on all indices when BQ-788 is also given suggests that the ETB receptor is crucial to the mechanism of this BQ-123-enalapril interaction. In an experimental rat model of interstitial renal fibrosis, enalapril treatment has been shown to increases ETB mRNA expression [411], providing a possible mechanism for this ET-1/ACE interaction. It is possible that ETA receptor antagonism then results in displacement of endogenous ET-1 from the ETA receptor onto the unblocked, upregulated ETB receptor.

As BQ-788 is a vasoconstrictor, it is possible that its ability to abolish the vasodilator effect of enalapril and BQ-123 is non-specific. However, the previous study in healthy volunteers and CRF patients (Chapter 6) has demonstrated that, while this dose of BQ-788 alone produces systemic and renal vasoconstriction, it does not abolish the vasodilator effects of BQ-123 suggesting the effect seen here is specific to the interaction between ACE inhibition and ETA receptor antagonism.

These studies were performed in subjects in a salt replete state (mean 24 hr urine excretion 119 ± 6mmol). A recent study has suggested that ET-1 plays a role in angiotensin-dependent hypertension in humans [412] particularly in respect of blood pressure and proteinuria. Salt depletion, with enhancement of renin-angiotensin activity, might therefore further enhance the synergy seen between ETA receptor antagonism and ACE inhibition.

In this study, in the presence of ACE inhibition, combined ETA/B receptor antagonism actually ERVR and ERBF, underlining the importance of this ETB mediated vasodilatation. This finding has important implications for the therapeutic use of ET receptor antagonists, suggesting that, in conjunction with ACE inhibitors,

ETA will be superior to ETA/B receptor antagonists in respect of haemodynamic benefits. This demonstration of an ETB receptor dependent, synergistic interaction in healthy subjects between ETA receptor antagonism and ACE inhibition suggests these differences observed in renal patients in chapter 5 may be, at least in part, due to concomitant chronic treatment with ACE inhibitors.

It is important to note that BQ-123 concentrations were higher when subjects were pre-treated with ACE inhibition. The number of subjects studied was small and this difference did not reach significance, but it is interesting to note that in Chapter 5, CRF patients also had higher BQ-123 plasma concentrations that healthy volunteers (again, however, not significant). In light of this, a pharmacokinetic interaction between enalapril and BQ-123 cannot be fully excluded without larger studies.

In summary, this mechanistic study demonstrates, in healthy men, that pre-treatment with ACE inhibition increases the systemic haemodynamic effects of ETA receptor antagonism and unmasks a renal haemodynamic and renal tubular effect, and that this synergy is requires an unblocked ETB receptor. These findings would support the use of ETA but not combined ETA/B receptor antagonists as useful adjunctive treatments to ACE inhibitors in the treatment of the systemic and renal vascular consequences of diseases characterised by vasoconstriction particularly in circumstances where sodium loss would also be beneficial such as heart failure or CRF. This interaction should now be explored by longer term studies in patients with such conditions.

Chapter 7

Mechanism of synergism between endothelin-A receptor antagonism and ACE inhibition in healthy volunteers

7.1 Introduction

ACE inhibitors, by preventing the breakdown of bradykinin [392], and ET-1, acting on endothelial ETB receptors [413], both increase endothelium-dependent vasodilatation. Having demonstrated a synergism between ETA receptor antagonism and ACE inhibitors that is dependent upon an unblocked ETB receptor (Chapter 6), the aim of this study was to explore the possible the mechanisms by which this interaction might occur. During co-administration of ACE inhibition and ETA receptor antagonism, subjects underwent selective blockade of NO production with L-NMMA and prostaglandin production with indomethacin.

7.2 Study design

This was a randomised, double-blind, placebo-controlled study 6 healthy subjects. For demographic data, see Table 7.1. Subjects attended for 4 visits, each separated by ≥7 days. For each visit, they received pre-treatment with enalapril. During the study day they then received placebo, BQ-123, BQ-123 + indomethacin or BQ-123 + L-NMMA, in a randomised order (Table 7.1). Comparisons of interest were pre-identified as placebo vs. BQ-123 and vs. BQ-123 + indomethacin/L-NMMA, and BQ-123 vs. BQ-123 + indomethacin/L-NMMA. This study ran concurrently with the study described in Chapter 8 (ie. 5 randomised visits in total). Placebo and BQ-123 phases are the same in the two studies.

Table 7.1 Subject demographic data

Age (yr)	47 ± 2 (30 -59)
Body mass index (kg/m^2)	23 ± 2 (18 - 29)
MAP (mmHg)	84.9 ± 3.1 (70.8 – 98.7)
Creatinine (μmol/L)	86 ± 8 (62 – 119)
Urinary Na excretion (mmol/24 hr)	103 ± 15 (61 – 162)
Cholesterol (mmol/L)	5.1 ± 0.3 (3.9 – 6.1)

For 5 days before each study, subjects took enalapril at a dose of 20 mg twice daily (see methods for justification of dosing schedule) taking the last dose at 0830 on the study day, along with indomethacin or a placebo capsule. Subjects then underwent a standard clearance study. After baseline measurements, the low dose of antagonist and L-NMMA or placebo was then administered followed by three 30 min collection periods. The higher dose of antagonist and L-NMMA or placebo was then administered followed by 5 further 30 min collection periods. To ensure blinding with ET-1 (Chapter 8 study), L-NMMA infusion was followed by saline for 55 min, and placebo was administered in 2 stages, over 5 min and 55 min after each dose of antagonist (Fig 8.1).

At 0, 60 & 90 min after the start of low and high dose antagonist, and at the end of the study, blood samples were taken for the measurement of plasma ET-1 concentrations. Serum ACE activity was determined at 0830 am and at 1430 (60 min after the start of the higher dose antagonist).

7.3 Results

One subject experienced an increase in mean arterial blood pressure of 50 mmHg following indomethacin administration and before receiving BQ-123, and was withdrawn from the study and replaced. Baseline measurements were not different in any phase of the study (Table 7.2).

7.3.1 Systemic haemodynamics (Fig 7.1)

The reduction in blood pressure observed after BQ-123 in the presence of enalapril (maximum placebo-corrected change from baseline –6.0 ± 1.7 mmHg, ANOVA $p<0.01$ vs. placebo) was abolished by L-NMMA (–2.0 ± 2.7 mmHg, $p<0.01$ vs. BQ-123+E), and moderately augmented by indomethacin, an effect most evident towards the end of the study (–11.0 ± 2.3 mmHg, $p<0.01$ vs. placebo and vs. BQ-123+E). SVRI was reduced by E+BQ-123 (–541 ± 119 dyne.s m^2/cm^5, $p<0.01$ vs. placebo), and HR and CI increased. These changes were unaffected by indomethacin

administration (SVRI –706 ± 148 dyne.s m^2/cm^5, $p<0.01$ vs. placebo), and abolished by L-NMMA (SVRI –194 ± 116 dyne.s m^2/cm^5, $p<0.01$ vs. BQ-123+E).

Table 7.2: Baseline data

	Enalapril + Placebo	Enalapril + BQ-123	Enalapril + BQ-123 + Indomethacin	Enalapril + BQ-123 + L-NMMA
MAP (mmHg)	82.4±2.6	84.2±3.5	90.7±1.8	80.5±2.4
SVRI (dyne.s m^2/cm^5)	2291±275	2340±279	2679±348	2108±281
CI (L/min/m^2)	3.1±0.4	3.0±0.3	2.9±0.4	3.3±0.3
HR (bpm)	59.6±2.8	60.1±3.2	54.7±2.3	60.1±2.3
ERBF (ml/min)	760±108	759±134	574±87	862±98
ERVR (mmHg.min/L)	124±22	126±18	177±25	105±18
EFF (%)	24±2	25±3	26±1	22±3
GFR (ml/min/1.73m^2)	106±7	106±12	93±13	110±12
UNaV (μmol/min)	132±16	121±21	60±16	124±24
Plasma ET-1 (pg/ml)	3.9±0.5	3.4±0.6	5.2±0.6	5.0±0.6

7.3.2 Renal haemodynamics (Fig 7.2)

The effects of BQ-123+E were abolished by NOS inhibition (ERBF: BQ-123+E 179 ± 63 ml/min; BQ-123+E+LNMMA –217 ± 98 ml/min, $p<0.01$ vs. BQ-123+E. ERVR: BQ-123+E –61 ± 23 mmHg.min/L; BQ-123+E+LNMMA –14 ± 26 mmHg.min/L, $p<0.01$ vs. BQ-123+E), but unaffected by indomethacin (ERBF: 121 ± 37 ml/min; ERVR: –42 ± 16 mmHg.min/L).

Figure 7.1 Systemic haemodynamics

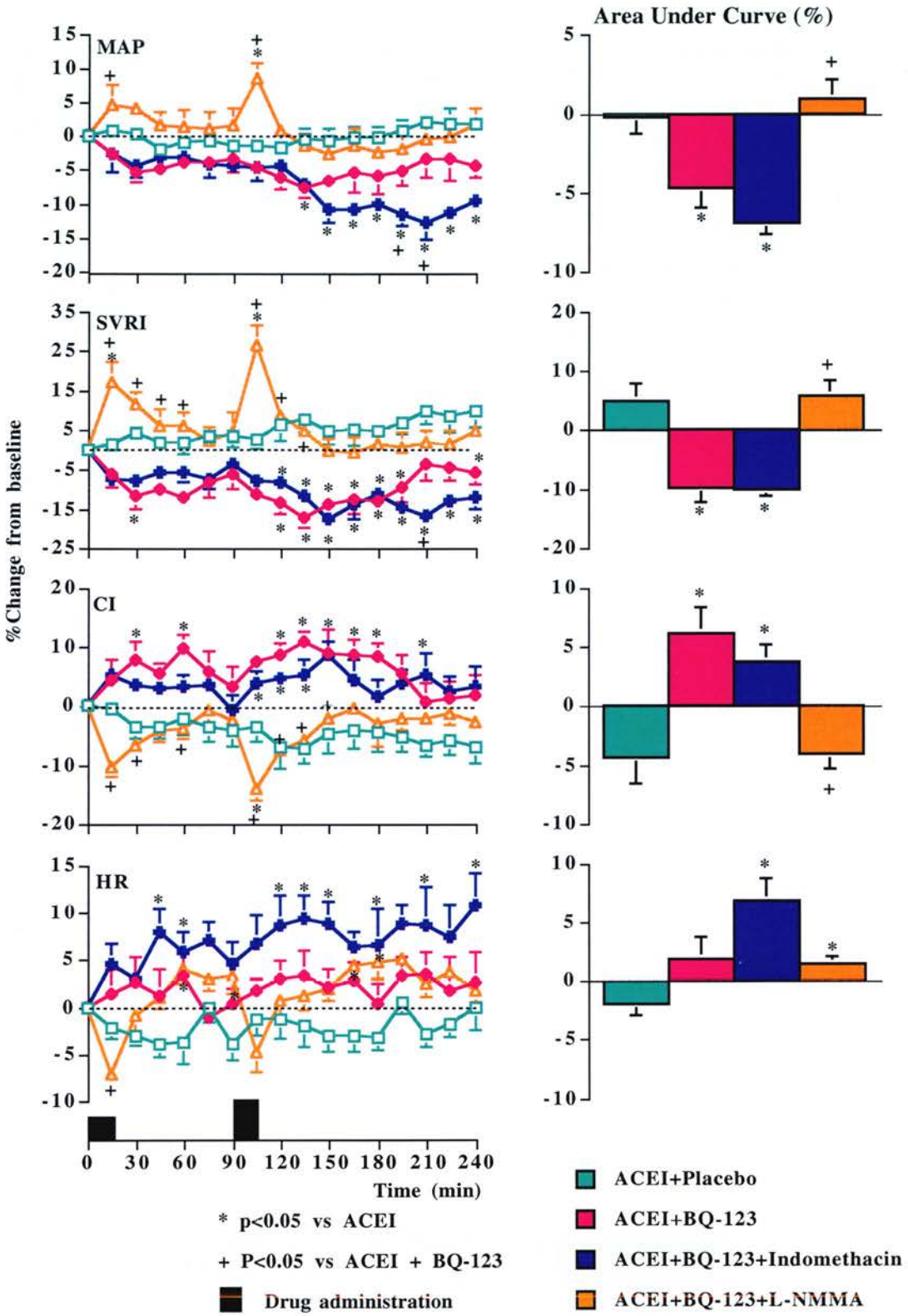
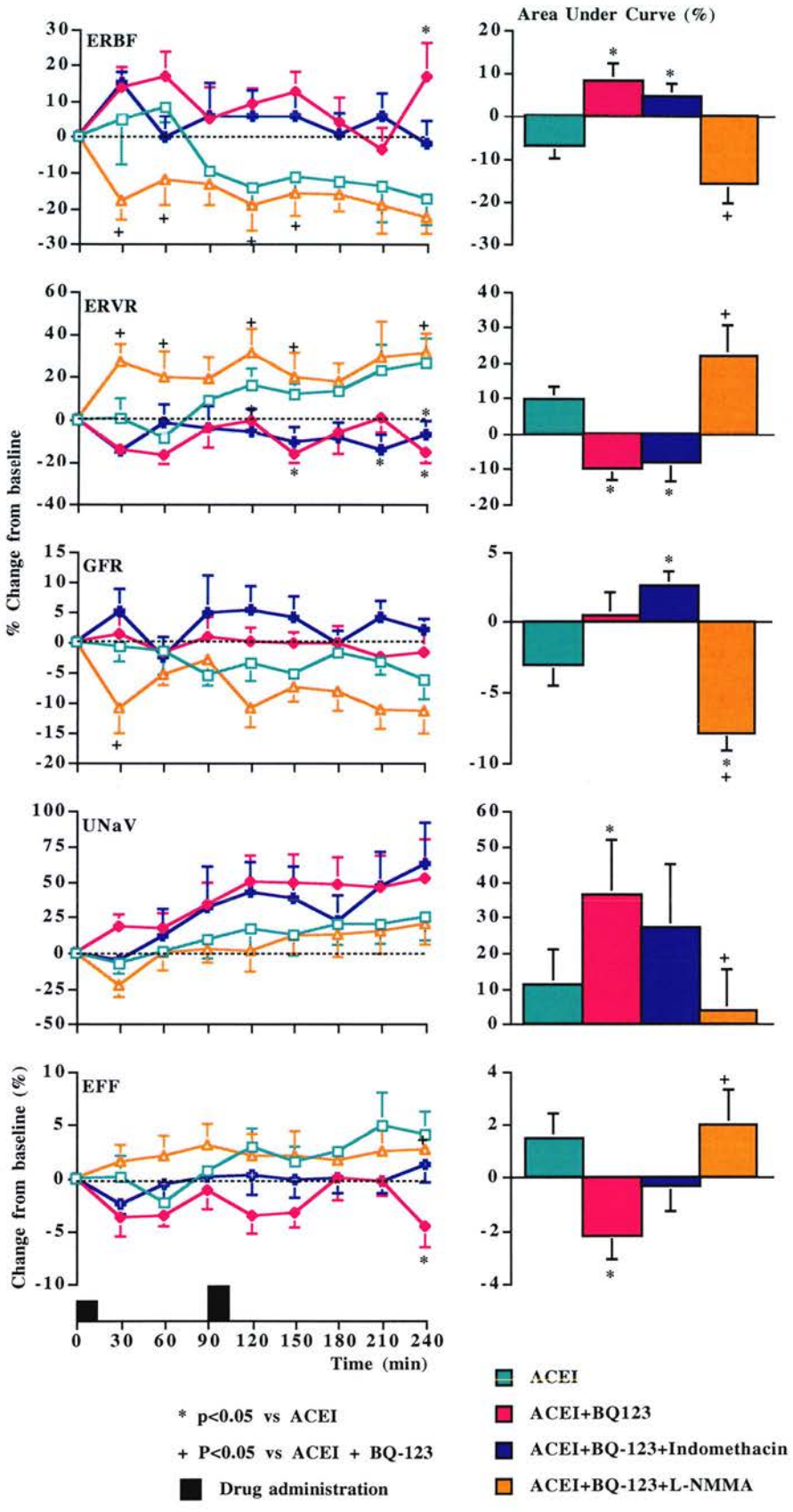


Figure 7.2 Renal indices



7.3.3 Urinary sodium excretion (Fig 7.2)

The natriuresis produced by the combination of BQ-123+E (UNaV: 29.3 ± 7.4 μmol/min) was abolished by L-NMMA (–27.4± 20.3 μmol/min, $p<0.01$ vs. BQ-123+E) and attenuated by indomethacin (–13.6 ± 11.7 μmol/min, $p<0.01$ vs. BQ-123+E).

7.3.4 Serum ACE activity

Serum ACE activity at t = 0 and 180 min confirmed inhibition of ACE at the start of and throughout each study day. There was no difference in ACE activity during any phase of the study (Table 7.3).

Table 7.3 Serum ACE activity (AU)

Serum ACE activity (AU)	E + Placebo	E + BQ-123	E + BQ123 + Indomethacin	E +BQ-123 + L-NMMA
t = 0 min	6.1±3.3	12.6±4.0	11.2±4.1	12.6±4.4
t = 180 min	12.4±4.9	4.8±2.0	5.7±3.0	2.1±2.1
0 vs. 180 min	p=0.36	p=0.15	p=0.34	p=0.09

7.3.5 Plasma ET-1 concentrations

Plasma ET-1 concentrations were not affected by pre-treatment with enalapril alone, or by subsequent treatment with BQ-123, indomethacin or L-NMMA (Table 7.4).

Table 7.4 Plasma ET-1 concentrations (pg/ml)

Plasma ET-1 (pg/ml)	E + Placebo	E + BQ-123	E + BQ123 + Indomethacin	E +BQ-123 + L-NMMA
t = 0 min	3.9±0.5	3.4±0.6	5.2±0.6	5.0±0.6
t = 120 min	3.8±0.5	4.7±0.3	4.5±0.7	5.1±0.5
ANOVA*	p=0.91	p=0.55	p=0.53	p=0.46

* for 0, 30, 90, 120, 180 & 240 min measurements

7.4 Discussion

This study has demonstrated that synergy between ACE inhibition and ETA receptor antagonism is abolished by NOS inhibition but largely unaffected by prostaglandin inhibition, leading us to conclude that ETA receptor antagonism and ACE inhibition act synergistically through an ETB receptor-mediated, NO-dependent and COX-independent mechanism. This systemic data is in keeping with previous forearm work demonstrating that the dilatory effects of BQ-123 are mediated through NO but not prostaglandins [194].

As L-NMMA is a vasoconstrictor, it is possible that its ability to abolish the vasodilator effect of enalapril and BQ-123 is non-specific. However, indomethacin has also been shown to produce renal vasoconstriction in healthy subjects [414] but it had no effect on the renal vasodilatation seen in this study after enalapril and BQ-123, suggesting the effect of L-NMMA seen here is specific to the interaction between ACE inhibition and ETA receptor antagonism.

While prostacyclin is the major COX product of macrovascular endothelium *in vitro*, COX activity can produce both vasoconstrictor and vasodilator arachadonic acid derivatives. Prostaglandins stimulate renin release [415]. Thus indomethacin may produce both vasodilatation, by inhibition of renin-mediated ANG II generation and COX-1-mediated thromboxane A2 synthesis, and vasoconstriction, by blocking synthesis of vasodilator prostanoids. Studies in animals also suggest that vasoconstrictor COX products, such as thromboxane A2 and prostaglandin H2, might be implicated in ET-1 induced vasoconstriction, particularly in disease models [416-420]. The greater fall in blood pressure when indomethacin was co-administered with enalapril and BQ-123 may represent blockade of the action of these constrictor COX products. Additionally, there is animal evidence that vasodilator prostaglandins may also be involved in the actions of ET in the renal

circulation, particularly the renal medulla [418, 421]. This study did not, however, demonstrate any inhibitory effect of indomethacin on the systemic and renal haemodynamic effects of the combination of ACE inhibition and ETA receptor antagonism. In respect of RBF, however, clearance studies only measure total RBF. It is possible, therefore, that opposite changes are occurring in the renal cortex and medulla.

In respect of natriuresis, knockout and antagonist studies in animals have implicated ETB receptors, in association with NO production, in ET mediated sodium excretion [135]. COX inhibition however, augments big ET mediated natriuresis, suggesting the involvement of prostaglandins, in an anti-natriuretic role [422]. This study demonstrates, in the presence of ACE inhibition, an ETB dependent natriuresis that is mediated by both NO and, to a lesser extent, prostanoids.

In summary, this mechanistic study demonstrates, in healthy men, that the systemic and renal haemodynamic synergy between ACE inhibition and ETA receptor antagonism operates largely via a NO dependent mechanism.

Chapter 8

Effects of exogenous ET-1 administration in the presence of ET-A receptor antagonism and ACE inhibition in healthy volunteers

8.1 Introduction

The ETB receptor appears to have a natriuretic role at least in animals. ETB knockout mice, and ETB antagonist treated rats, develop a sodium dependent hypertension [134, 135]. Dogs subjected to high-grade ETA blockade demonstrate a vasodilatation and natriuresis in response to administration of exogenous low dose ET-1[123]. However, ET-1/ETB induced natriuresis has not been demonstrated in man. In this study exogenous ET-1 was administered in the presence of ACE inhibition and ETA blockade to examine the effects of ETB receptor activation on systemic and renal haemodynamics

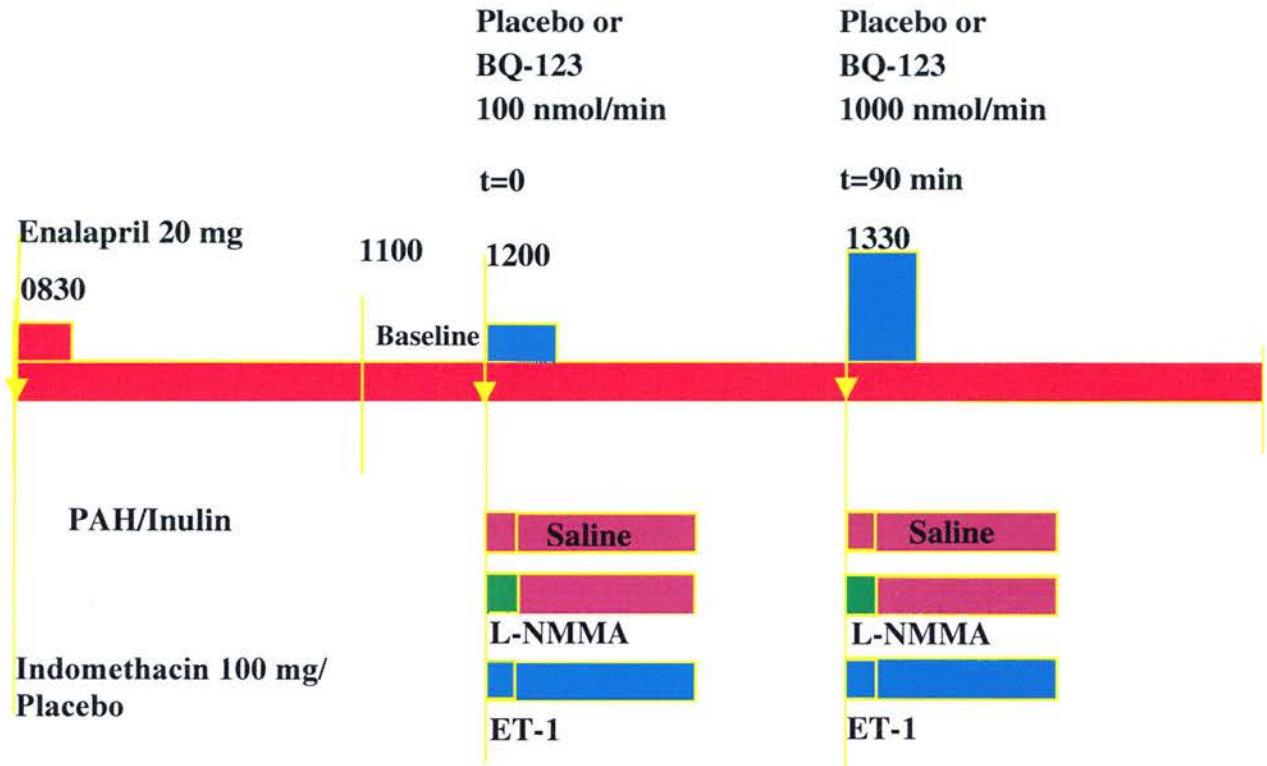
8.2 Study design

This was a randomised, double-blind, placebo-controlled study 6 healthy subjects. Subjects attended for 3 visits, each separated by ≥7 days. For each visit, they received pre-treatment with enalapril. During the study day they then received placebo, BQ-123, or BQ-123 + ET-1 in a randomised order (Fig 8.1). Comparisons of interest were pre-identified as placebo vs. BQ-123 and vs. BQ-123 + ET-1, and BQ-123 vs. BQ-123 + ET-1. This study ran concurrently with the study described in Chapter 7 on the same subjects (ie. 5 randomised visits in total). Placebo and BQ-123 phases are the same in the two studies.

For 5 days before each study, subjects took enalapril at a dose of 20 mg twice daily (see methods for justification of dosing schedule) taking the last dose at 0830 on the study day. At 0830 they also received a placebo capsule to allow indomethacin administration (Chapter 7 study) to be blinded. Subjects then underwent a standard clearance study. After baseline measurements, the low dose of antagonist was administered over 15 min and ET-1 or placebo over 60 min followed by three 30 min collection periods. The higher dose of antagonist and ET-1 or placebo was then administered followed by 5 further 30 min collection periods. To ensure blinding for L-NMMA administration (Chapter 7 study), ET-1 and saline placebo were

administered in 2 stages, over 5 min and 55 min after each dose of antagonist (Fig 8.1).

Fig 8.1 Study protocol (Chapter 7&8)



At 0, 60 & 90 min after the start of low and high dose antagonist, and at the end of the study, blood samples were taken for the measurement of plasma ET-1 concentrations. BQ-123 plasma concentrations were measured before and at 15, 45 & 90 minutes after the start of each dose of antagonist, and at the end of the study. Serum ACE activity was determined at 0830 am and at 1430 (60 min after the start of the higher dose antagonist).

8.3 Results

All subjects completed the study without adverse effects. Baseline measurements were not different in any phase of the study (Table 8.1).

Table 8.1 Baseline data

	E+Placebo	E+BQ-123	E+BQ-123+ET-1
MAP (mmHg)	82.4±2.6	84.2±3.5	82.1±3.9
SVRI (dyne.s m^2/cm^5)	2291±275	2340±279	2238±297
CI (L/min/m^2)	3.1±0.4	3.0±0.3	3.1±0.2
HR (bpm)	59.6±2.8	60.1±3.2	61.9±2.9
ERBF (ml/min)	760±108	759±134	675±69
ERVR (mmHg.min/L)	124±22	126±18	132±14
EFF (%)	24±2	25±3	24±2
GFR (ml/min/1.73m^2)	106±7	106±12	102±8
UNaV (μmol/min)	132±16	121±21	137±36
Plasma ET-1 (pg/ml)	3.9±0.5	3.4±0.6	5.5±0.7

8.3.1 Systemic haemodynamics (Fig 8.2)

The reduction in blood pressure observed after BQ-123 in the presence of enalapril (maximum placebo-corrected change from baseline –6.0 ± 1.7 mmHg) was abolished by ET-1 (–6.0 ± 1.7 mmHg). SVRI was reduced by E+BQ-123, and HR and CI increased. Administration of exogenous ET-1 abolished all systemic haemodynamic changes produced by BQ-123 except for the increase in HR.

Figure 8.2 Systemic haemodynamics

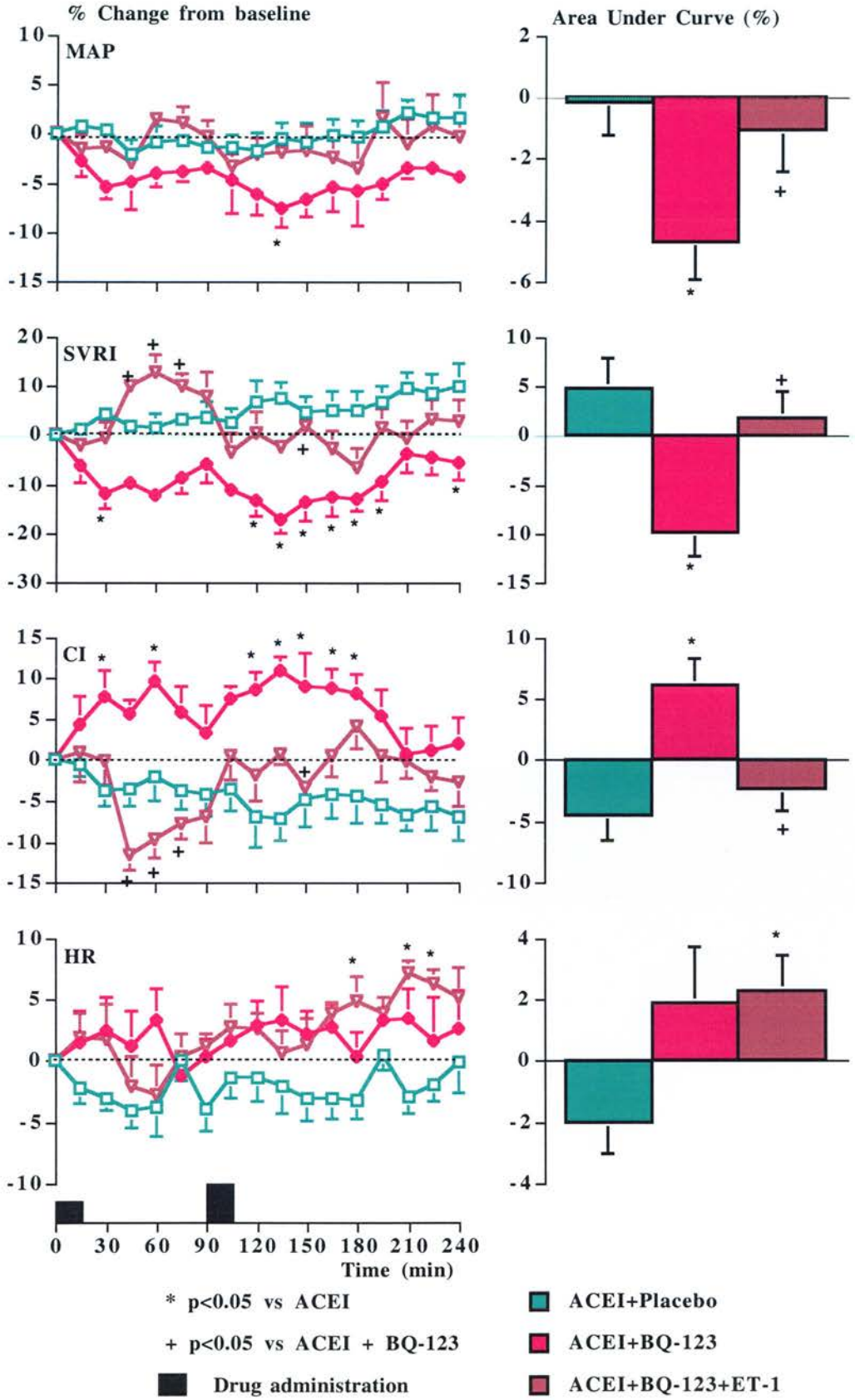
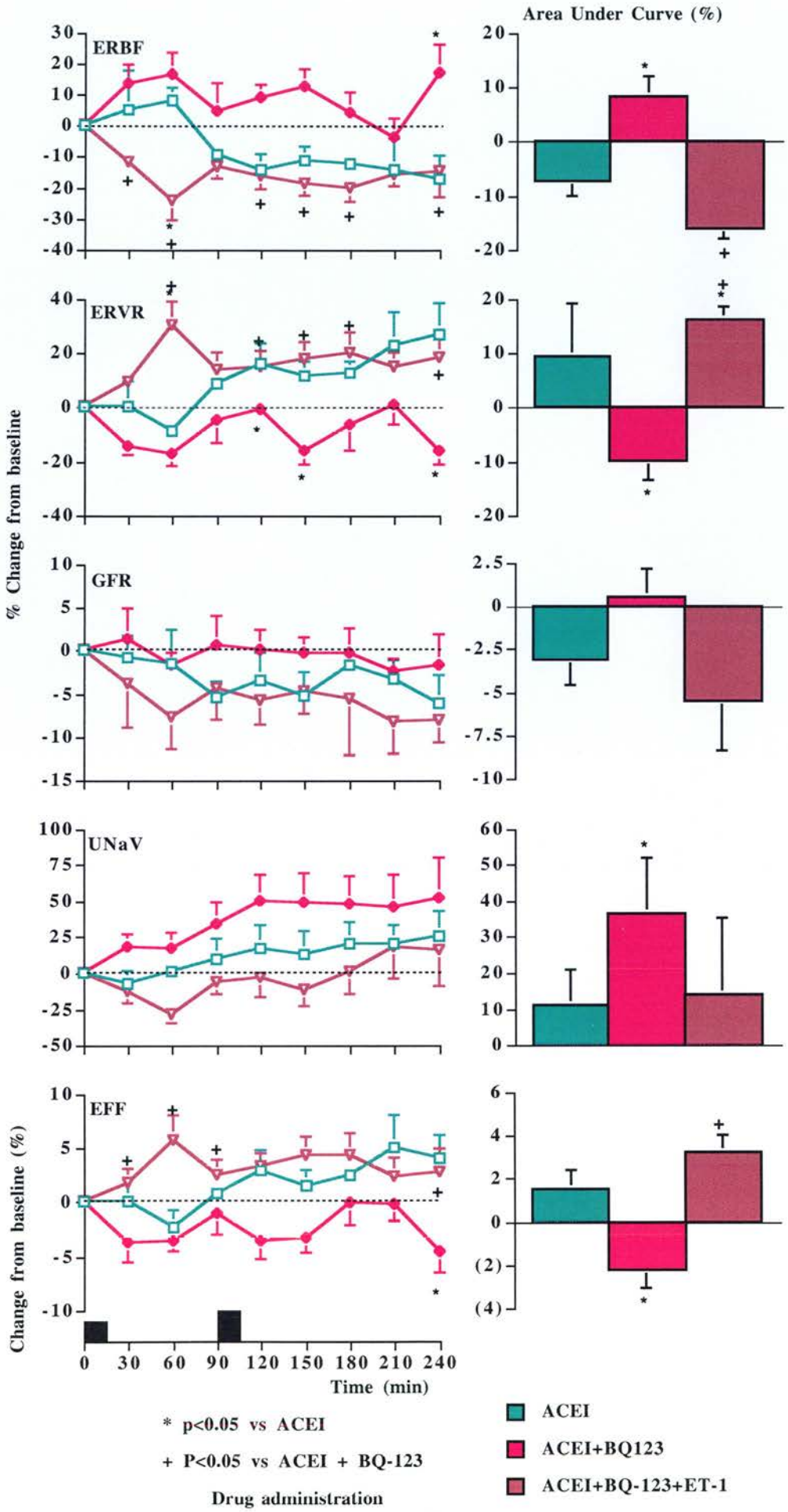


Figure 8.3 Renal indices



8.3.2 Renal haemodynamics (Fig 8.3)

ET-1 administration abolished all renal haemodynamic changes produced by BQ-123. (ERBF: BQ-123+E 179 ± 63 ml/min; BQ-123+E+ET-1 –213 ± 56 ml/min, $p<0.01$ vs. BQ-123+E. ERVR: BQ-123+E –61 ± 23 mmHg.min/L; BQ-123+E+ET-1 +54 ± 1.5 mmHg.min/L, $p<0.01$ vs. BQ-123+E).

8.3.3 Urinary sodium excretion (Fig 8.3)

The natriuresis produced by the combination of ACE inhibition and ETA receptor antagonism (UNaV: 29.3 ± 7.4 μmol/min) was abolished by exogenous ET-1 infusion (UNaV: -38.2±23.6, $p<0.01$ vs. BQ-123+E).

8.3.4 Serum ACE activity

Serum ACE activity at t = 0 and 180 min confirmed inhibition of ACE at the start of and throughout each study day. There was no difference in ACE activity during any phase of the study (Table 8.2).

Table 8.2 Serum ACE activity (AU)

Serum ACE activity (AU)	E + Placebo	E + BQ-123	E + BQ-123 + ET-1
t = 0 min	6.1±3.3	12.6±4.0	6.2±3.1
t = 180 min	12.4±4.9	4.8±2.0	5.6±2.5
0 vs. 180 min	p=0.36	p=0.15	p=0.76

8.3.5 Plasma ET-1 concentrations

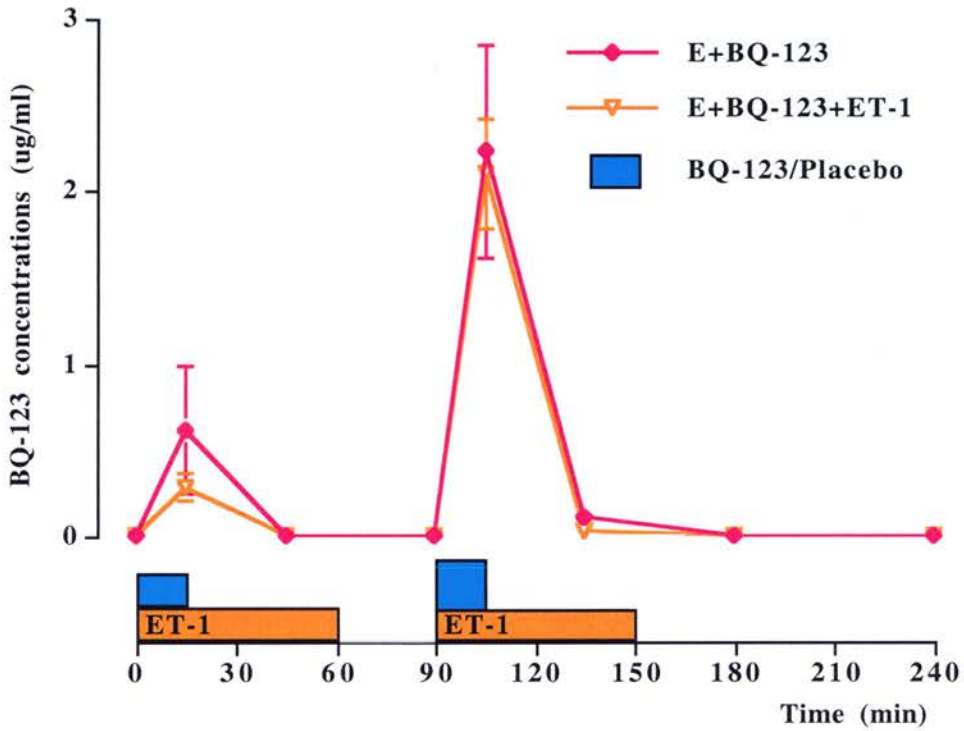
Administration of exogenous ET-1 resulted in a threefold rise in plasma ET-1 concentrations 30 min after commencing each ET-1 infusion (t=0 min, 5.5±0.7 pg/ml; t=30 min, 12.5±2.8 pg/ml; t=120 min, 15.1±3.6 pg/ml, $p<0.05$ vs. placebo).

Plasma concentrations had reduced back to baseline 90 min after commencing ET-1 infusion (t=90 min, 5.4±0.5 pg/ml; t=180 min, 6.5±0.8 pg/ml)

8.3.6 Plasma BQ-123 (Fig 8.4)

Plasma BQ-123 concentrations were not altered by infusion of exogenous ET-1

Figure 8.4 Plasma BQ-123 levels ± exogenous ET-1 infusion



8.4 Discussion

This study demonstrates that that ET-1 abolishes the systemic and renal vasodilatation and natriuresis produced by combined ACE inhibition and ETA receptor antagonism.

Studies in dogs have used ETA receptor blockade to unmask a renal vasodilatory and natriuretic effect of ET-1, presumed to be due to ETB receptor activation [123]. In administering exogenous ET-1 during combined ACE inhibition and ETA receptor blockade, the aim had been to demonstrate an effect of direct ETB receptor stimulation in man. However, administration of exogenous ET-1 in this study served only to abolish the systemic and renal effects of combined ACE inhibition and ETA receptor blockade. BQ-123 is a competitive antagonist at the ETA receptor [93] whereas ET-1 is a non-competitive agonist [413]. It would be important therefore to ensure that plasma ET-1 concentrations do not achieve levels sufficient to displace BQ-123 from the ETA receptor. A previous study has demonstrated that an equivalent dose of BQ-123 to our low dose is sufficient to abolish the systemic and renal vasoconstriction produced by this dose of ET-1 [139]. Therefore, in designing the study, it was hoped that the high dose of BQ-123, an order of magnitude greater, would be sufficient to sustain ETA blockade in the presence of this dose of ET-1. The abolition of vasodilatation and natriuresis does suggest that a degree of ETA receptor activation is occurring by displacement of BQ-123 by exogenous ET-1.However, no increases in plasma BQ-123 concentrations were observed in the presence of ET-1 to suggest any displacement from its receptor. It is possible, therefore, that the ETB receptor does not play a role in renal vasodilatory tone and sodium regulation in healthy volunteers. However, all studies so far presented (Chapter 5 & 6) and previous work from the department [194, 210] suggests that the net result of ETB receptor activation is to produce vasodilatation in man. This needs to be clarified using lower doses of ET-1 in the presence of high grade ETA receptor blockade.

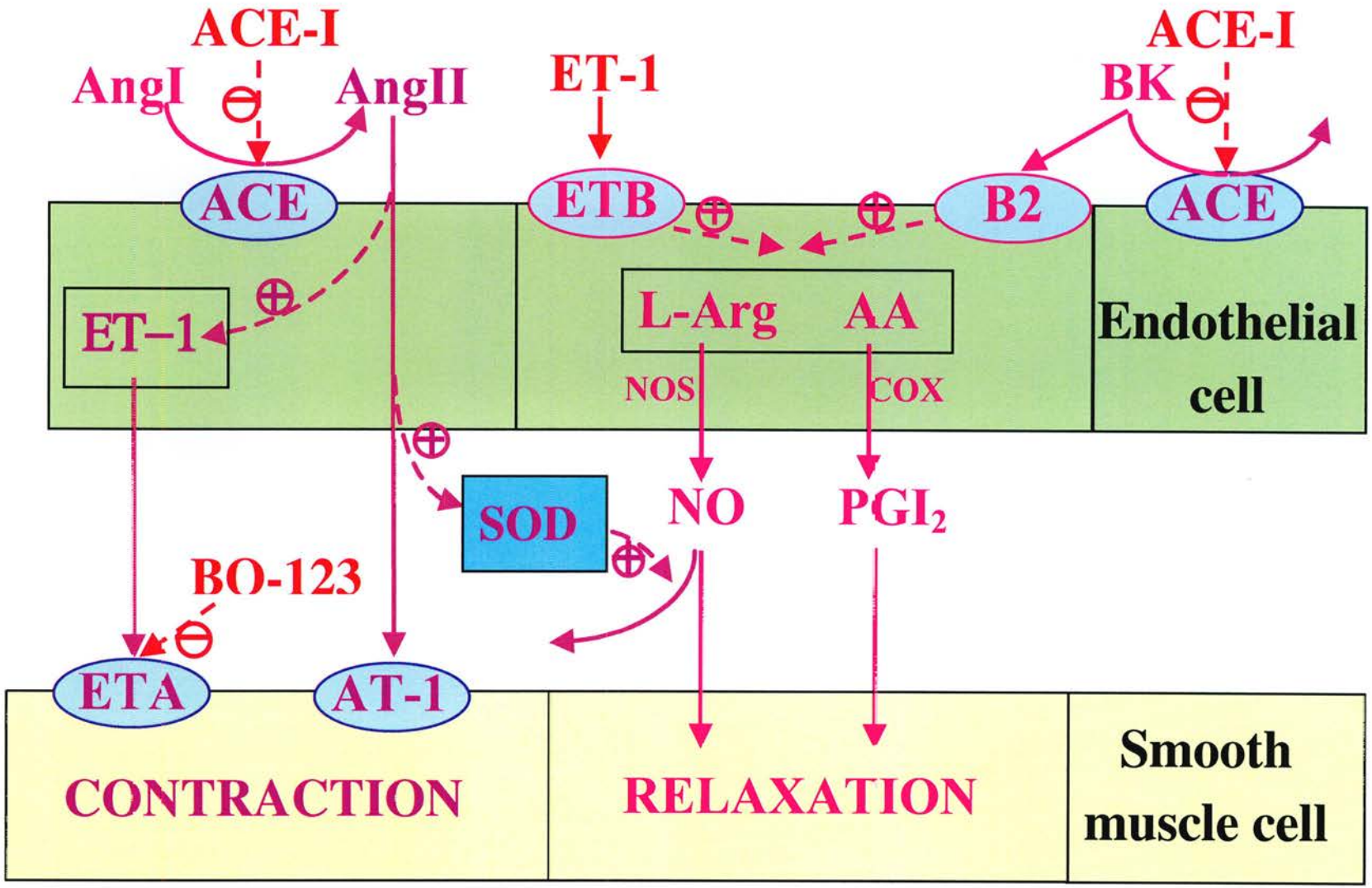


Figure 8.1: ET-1 administration under conditions of ACE & ETA blockade

Key: Study drugs Inhibited Activated

Chapter 9

The systemic haemodynamic and renal response to acute ETA antagonism after a high salt intake in healthy volunteers

9.1 Introduction

Animal models of salt-sensitive hypertension have suggested an important pathogenic role for ET-1 [423] and the therapeutic potential for ETA receptor antagonism in this condition [424-426]. In man, plasma and urinary ET concentrations have been shown to be elevated in salt-sensitive individuals [427, 428], though this is not a uniform finding [215].

A high salt intake has also been shown to increase vascular NO activity in man [429] and the response to NOS inhibition increases on a high salt diet suggesting a greater dependence on the dilatory actions of NO when salt loaded [429, 430]. This upregulation of NO is likely to represent an important adaptation to increased salt intake and studies suggest that a relative deficiency of endothelial NO activity may contribute to the development of salt-sensitive hypertension [426, 431, 432].

ETA antagonism with BQ-123 decreases vascular tone in healthy volunteers and patient groups (Chapters 3 & 5). Forearm studies suggest these dilator effects are largely mediated though nitric oxide (NO) [194]. The hypothesis for this study was, therefore, that in individuals whose blood pressure exhibited a high degree of salt sensitivity, the relative reduction in endothelial NO activity would result in a reduced systemic haemodynamic response to BQ-123 compared to salt-resistant individuals.

9.2 Study design

This was a randomised, double-blind, placebo-controlled study 6 healthy subjects. For demographic data, see Table 9.1. Subjects attended for 4 visits, each separated by $\geq$7 days receiving placebo or BQ-123 in a randomised order in the presence or absence of sodium supplementation. For 3 days before each study subjects adhered to a standardised diet restricting salty foods (restricted salt: RS). On two of the four visits, subjects also took 15 slow sodium tablets a day (10 mmol each) to achieve a high salt (HS) intake. Comparisons of interest were pre-identified as placebo vs. BQ-123 on a RS diet, placebo vs. BQ-123 on a HS diet and BQ-123 on a RS vs. a HS diet. Salt sensitivity

index (SSI) was calculated as the percentage difference between baseline MAP on a high salt compared to a routine salt diet.

Table 9.1 Subject demographic data

Age (yr)	51±3 (41 - 64)
Body mass index (kg/m^2)	24 ± 2 (19 - 33)
MAP (mmHg)	98.2 ± 8.8 (88.6 – 108.5)
Creatinine (μmol/L)	93 ± 8 (62 - 119)
Cholesterol (mmol/L)	5.2±0.2

On each study day, subjects underwent a standard clearance study. After baseline measurements, the low dose of BQ-123 was administered followed by three 30 min collection periods. The higher dose of BQ-123 was then administered followed by 5 further 30 min collection periods.

At 0, 60 & 90 min after the start of low and high dose of BQ-123, and at the end of the study, blood samples were taken for the measurement of plasma ET-1, and urine samples for the measurement of urinary ET-1 excretion rates.

9.3 Results

One subject was unable to tolerate salt tablets, withdrew from the study and was replaced. Six subjects completed the study without adverse events. 24 hr urinary sodium excretion on the third day of a RS diet was 130±19 mmol/24 hrs. After the HS diet, this increased to 279±36 mmol/24 hrs ($p<0.01$ vs. RS). Baseline data are shown in Table 9.2.

Blood pressure at baseline was higher after the HS diet (RS both visits: 93.2±2.2, HS both visits: 101.0±3.2 $p<0.01$) but ERBF and GFR were unchanged. Plasma ET-1 concentrations were not increased (RS both visits: 4.8±0.3 pg/ml, HS both visits: 5.1±0.2 pg/ml) but urinary ET-1 excretion rates were higher after the HS diet (RS both visits: 10.6±1.2 pg/min, HS both visits: 13.7±1.4 pg/min $p<0.05$).

Table 9.2 Baseline data on routine salt (RS) vs. high salt (HS) diet

	Placebo RS diet	BQ-123 RS diet	Placebo HS diet	BQ-123 HS diet	RS vs. HS *t-test*
MAP (mmHg)	94.5±2.8	93.4±3.7	100.7±4.9	101.4±4.6	p<0.01
SVRI (dyne.s m^2/cm^5)	2527±297	2842±453	3009±512	2787±385	NS
CI ($L/min/m^2$)	3.2±0.4	2.9±0.4	3.0±0.4	3.1±0.4	NS
HR (bpm)	60.8±0.9	58.4±1.1	55.3±1.4*	55.6±1.3*	p<0.01
ERBF (ml/min)	695±78	703±75	732±118	737±96	NS
ERVR (mmHg.min/L)	150±13	144±17	159±32	153±25	NS
EFF (%)	24±2	22±2	23±2	22±2	NS
GFR ($ml/min/1.73m^2$)	99±11	102±13	105±10	104±10	NS
UNaV (μmol/min)	165±11	166±25	253±17	221±24	p<0.01
Plasma ET-1 (pg/ml)	4.7±0.5	4.9±0.4	5.2±0.2	5.0±0.2	NS
Urinary ET-1 excretion (pg/min)	11.3±1.5	10.0±1.8	12.3±2.2	15.1±1.5	p<0.05

*p<0.05 vs. Placebo RS diet

9.3.1 Systemic haemodynamics (Figs 9.1 & 9.2)

BQ-123 significantly reduced MAP and SVRI, and increased HR and CI on both a RS and a HS diet to an equal degree (p<0.01 vs. placebo) (Fig 9.1). However, the increase in baseline MAP on a high salt diet for each individual (SSI) did correlate with the extent to which he responded to ETA antagonism (R^2=0.76 p=0.02) (Fig 9.2). There was no correlation observed between the reduction in MAP after BQ-123 and baseline MAP (R^2<0.001 p=0.98), or 24 hour urinary sodium excretion (R^2<0.001 p=0.99) for that study day.

Figure 9.1 Systemic haemodynamics

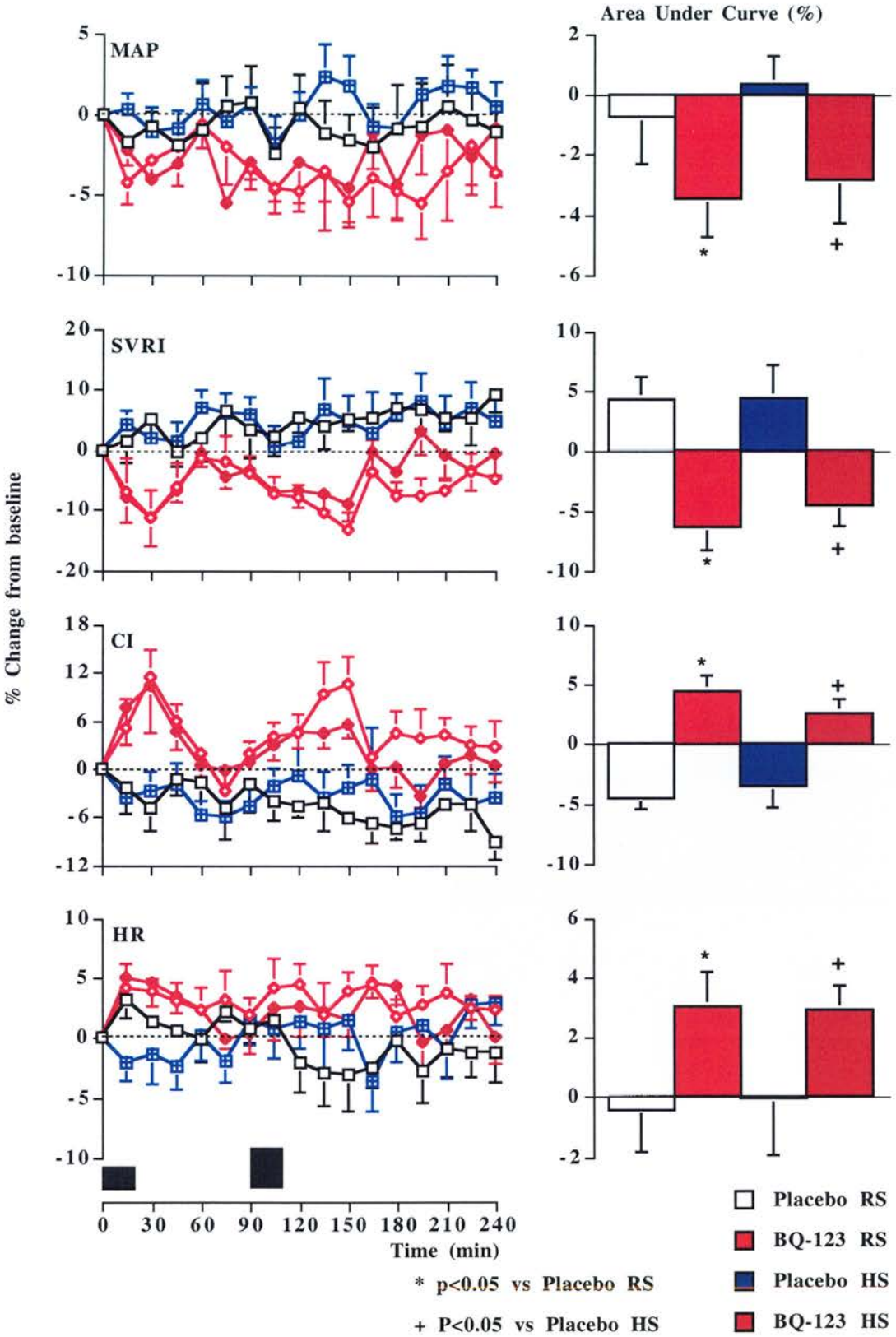
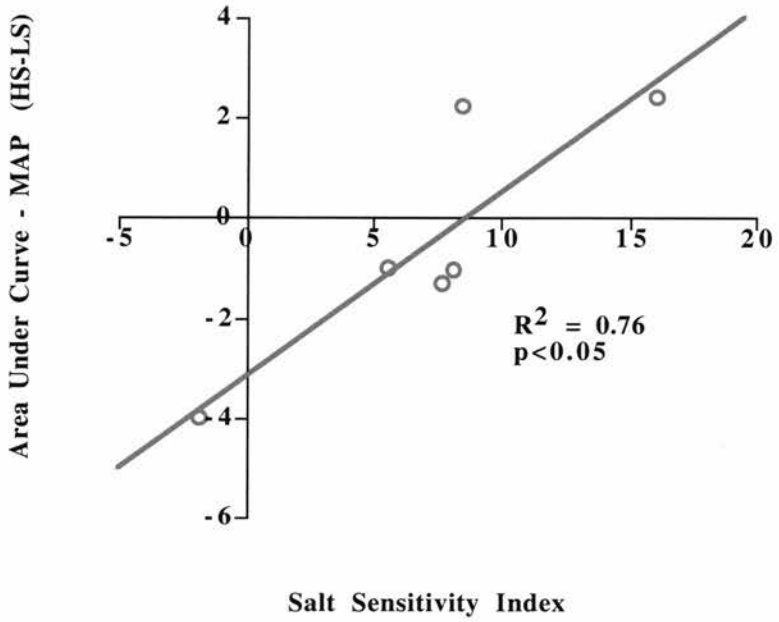


Figure 9.2 Salt sensitivity index vs response to BQ-123 - MAP



9.3.2 Renal haemodynamics (Figs 9.3 & 9.4)

Consistent with previous studies in healthy volunteers (Chapter 5) BQ-123 did not significantly alter ERBF, ERVR or GFR and no correlations were noted between SSI and ERVR (R^2=0.59 p=0.27), or ERBF (R^2=0.28 p=0.08).

9.3.3 Urinary sodium excretion

While BQ-123 modestly increased urinary sodium excretion on both diets (RS: +7.9±11.1%; HS: +10.7±12.0%, p<0.05 vs. HS placebo), there was no correlation between SSI and sodium excretion (R^2<0.001 p=1.00), or fractional excretion (R^2<0.001 p=0.96).

Figure 9.3 Salt sensitivity index vs response to BQ-123 - ERBF

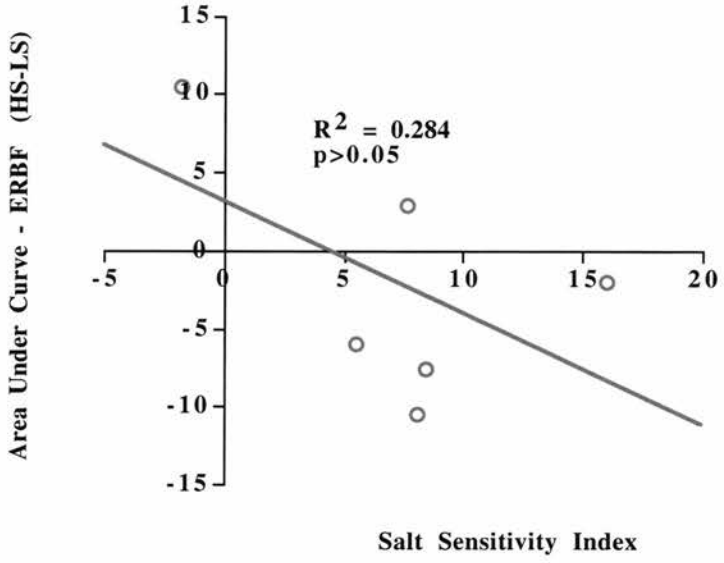
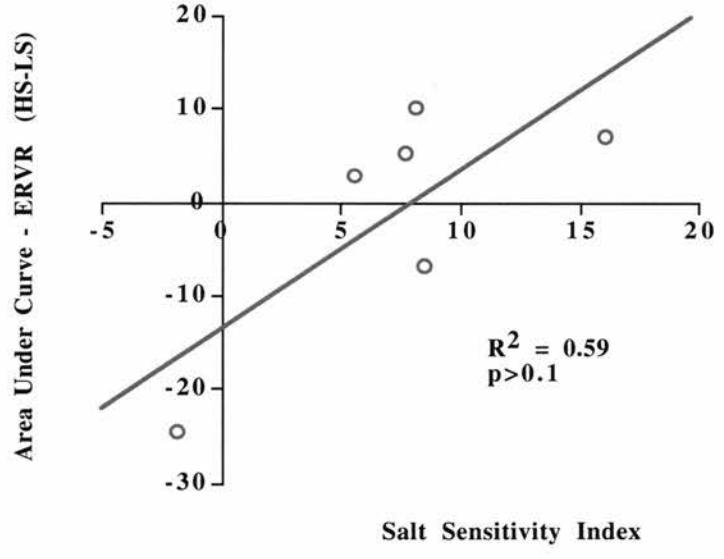


Figure 9.4 Salt sensitivity index vs response to BQ-123 - ERVR



9.3.4 ET-1 concentrations

Plasma ET-1 concentrations were not altered by any treatment (Table 9.3). Urinary ET-1 excretion rates were higher at baseline on a HS diet (Table 9.2) but did not

correlate with 24hr urinary sodium excretion or SSI. Urinary ET-1 excretion was not altered by administration of placebo or BQ-123 on either diet (Table 9.3).

Table 9.3 Plasma ET-1 concentrations (pg/ml) and urinary excretion rates (pg/min)

Plasma ET-1 concentrations (pg/ml)	Placebo RS diet	BQ-123 RS diet	Placebo HS diet	BQ-123 HS diet
t = 0 min	4.7±0.5	4.9±0.4	5.2±0.2	5.0±0.2
t = 120 min	4.8±0.5	4.1±0.3	4.9±0.3	5.1±0.5
ANOVA*	p=0.97	p=0.41	p=0.53	p=0.99
Urinary ET-1 excretion (pg/min)	**Placebo RS diet**	**BQ-123 RS diet**	**Placebo HS diet**	**BQ-123 HS diet**
t = 0 min	11.3±1.5	10.0±1.8	12.3±2.2	15.1±1.5
t = 120 min	10.2±1.2	11.7±2.5	9.8±1.3	14.2±1.6
ANOVA*	p=0.85	p=0.91	p=0.72	p=0.72

* for 0, 30, 90, 120, 180 & 240 min measurements

9.4 Discussion

While this study did not demonstrate a difference, in the group as a whole, in the effect of BQ-123 on systemic and renal haemodynamics on a HS diet compared to the RS diet, individuals with higher degrees of salt sensitivity of their blood pressure appear to have a blunted blood pressure response to ETA receptor antagonism on a high salt intake. This suggests that ETA antagonists would be more effective as treatments for hypertension in salt-sensitive individuals when such patients are salt restricted.

The role of ET in the response to salt and salt-sensitivity has been supported by a series of studies. Salt loading causes rats to develop greater hypertension on a high salt diet under conditions of chronic ETB receptor blockade [433], and the DOCA-salt hypertensive rat, a salt-sensitive strain demonstrates increased ET-1 production,

upregulation of ETB receptors and hypertension that is blocked by ETA receptor antagonism [434, 435]. Renal ET-1 in particular is important in the response to salt. Urinary ET-1 is increased by a high salt diet [433], and a high salt intake also augments the ETB receptor mediated medullary vasodilatation induced by big-ET-1 [133]. A high salt intake is known to increase renal NO activity [430]. However, salt-sensitive rats fail, unlike salt-resistant strains, to increase renal NOS activity in response to salt loading [426]. Taken together, these studies suggest that the response to an increased salt ingestion is to increase in ETB and NO mediated vasodilatation particularly in the renal medulla where it promotes natriuresis (that may be in part related to this increase in blood flow or direct tubular effect) as an adaptive response to limit hypertension, and that in salt-sensitive individuals, there is a failure to increase NO sufficiently and a consequent reduced sodium excretion and increased systemic vasoconstriction.

It has previously been demonstrated in forearm studies that ETA receptor antagonists achieve their haemodynamic effects through NO [194], presumed due to binding of ET-1 under conditions of ETA receptor blockade to the ETB receptor. It is possible that the lesser response to BQ-123 in salt-sensitive subjects seen in this study is a consequence of reduced endothelial NO activity in such individuals compared to salt-resistant individuals. However, if the lesser response to BQ-123 in salt-sensitive subjects is a consequence of reduced endothelial NO activity, concomitant ETB blockade will be less important in salt-sensitive individuals on a high-salt diet with loss of endothelial NO. This must be borne in mind in comparing the clinical effects of selective ETA and combined ETA/B receptors in unscreened subjects, as a high salt diet in salt-sensitive individuals may limit any differences between these two therapeutic approaches.

In this study, a high salt diet increased baseline blood pressure without an alteration in plasma ET-1 concentrations to suggest an upregulation of the ET system. However, due to the largely paracrine action of vascular endothelial ET-1, plasma ET-1 is not a good marker of the activity of the vascular ET system. Urinary ET-1, a marker of increased renal production [436], was, however, increased on a high salt diet consistent with increased renal ET-1 in response to salt loading. Despite this, no clear changes in renal blood flow or vascular tone could be demonstrated in this study either in the group as a

whole, or relating the changes observed in individuals to their salt-sensitivity, though the subject with the highest degree of salt-sensitivity did exhibit the greatest increase in ERVR and fall in ERBF on a high salt diet. PAH clearances can only measure total renal blood flow so changes in medullary or cortical blood flow may be missed. Nevertheless, SSI, however, had no influence on sodium excretion either in this study.

However, this study is limited by the small number of subjects studied and the fact that 4 out of the 6 subjects had very similar and small increases in blood pressure in response to increased salt intake. While a trend was observed in respect of a correlation between SSI and ERBF and ERVR, this did not reach significance. Further studies are needed in healthy volunteers and patients with a range of SSIs, particularly subjects with a high degree of salt sensitivity to explore this further.

In conclusion, the effectiveness of ETA receptor blockade may be limited in salt-sensitive individuals who continue to take a high salt diet. Identification of such individuals is therefore important, as pharmacological intervention for hypertension must be instituted in conjunction with dietary advice.

Chapter 10

Urinary excretion of endothelin-1

10.1 Introduction

ET-1 is largely removed from the circulation by receptor (ETB) mediated mechanisms, primarily in the pulmonary circulation [86-88]. Renal clearance, however, does account for ~10% of plasma ET-1 clearance in man [86]. ET-1 is a small polypeptide and plasma ET-1 will thus be filtered at the glomerulus, and, because it is dependent upon filtration, this renal clearance does not require receptor-mediated mechanisms. After filtration, in common with most polypeptides, ET-1 is likely to be hydrolysed at the brush border and the constituent peptides and amino acids reabsorbed in the tubules and metabolised. In rats, though up to 15% of plasma ET-1 is extracted across the renal bed [88] less than 1% of injected radiolabelled ET-1 is recovered in the urine [92]. The failure of plasma ET-1 in these isotope studies to appear in the urine implies that neither glomerular filtration nor tubular secretion of plasma ET-1 accounts for urinary ET-1, which is therefore assumed to be primarily of renal origin, and, hence, independent of plasma ET-1 concentrations.

In CRF, the consequence of a reduced filtration rate is a reduced filtered load of ET-1 delivered to the tubules. Thus renal ET-1 clearance will fall as renal function is lost, leading to increases in plasma concentrations of ET-1 [216]. Renal ET-1 production, and hence urinary ET-1 excretion, is, however, also increased in CRF [92, 188]. Therefore, while plasma ET-1 concentrations and urinary ET-1 excretion should be independent of each other [436], both will correlate inversely with GFR, and may, thus, appear to correlate with each other.

There is also recent animal evidence to suggest that ETB receptors may participate in the movement of proteins across the renal tubular epithelium [437]. Peri-tubular ET-B receptors are abundant in the human kidney [52]. However, their possible role in urinary ET-1 excretion has not been studied.

Using paired plasma and urine samples from the studies described in studies 3-7 (Chapter 5-9), this chapter compares plasma ET-1 concentrations and ET-1 excretion rates to establish if any relationship exists between these two measurements. Additionally, using measurements from study 3 (Chapter 5), the effect of ET receptor blockade on urinary excretion of ET-1 has been examined.

10.2 Protocol

During all of the clearance studies (studies 3-7), paired plasma and urine samples were taken at 0, 60 & 90 min after the start of both low and high dose antagonist administration, and at the end of the study, for the measurement of plasma and urinary ET-1 concentrations. Urinary excretion rate was calculated by multiplying urinary ET-1 concentration by urine flow rate.

10.3 Results

10.3.1 Correlation of plasma and urinary ET-1

Analysing the results from study 3 (Chapter 5) in isolation, at baseline (t=0), before administration of ET receptor antagonist, a limited correlation between plasma and urinary ET-1 excretion was observed (R^2=0.09, p=0.02). However, both plasma ET-1 concentration and urinary ET-1 excretion correlated inversely with baseline GFR (Fig 10.1). Fractional excretion of ET-1 also correlated inversely with baseline GFR (R^2=0.44, p<0.01) and only exceeded unity in patients with CRF (Fig 10.2).

Analysis of paired plasma and urine samples from all the phases of study 3 (384 paired samples) did not demonstrate a relationship between plasma ET-1 concentration and urinary ET-1 excretion (R^2=0.001, p=0.50) (Fig 10.3).

Figure 10.1 Plasma & urinary ET-1 in baseline samples

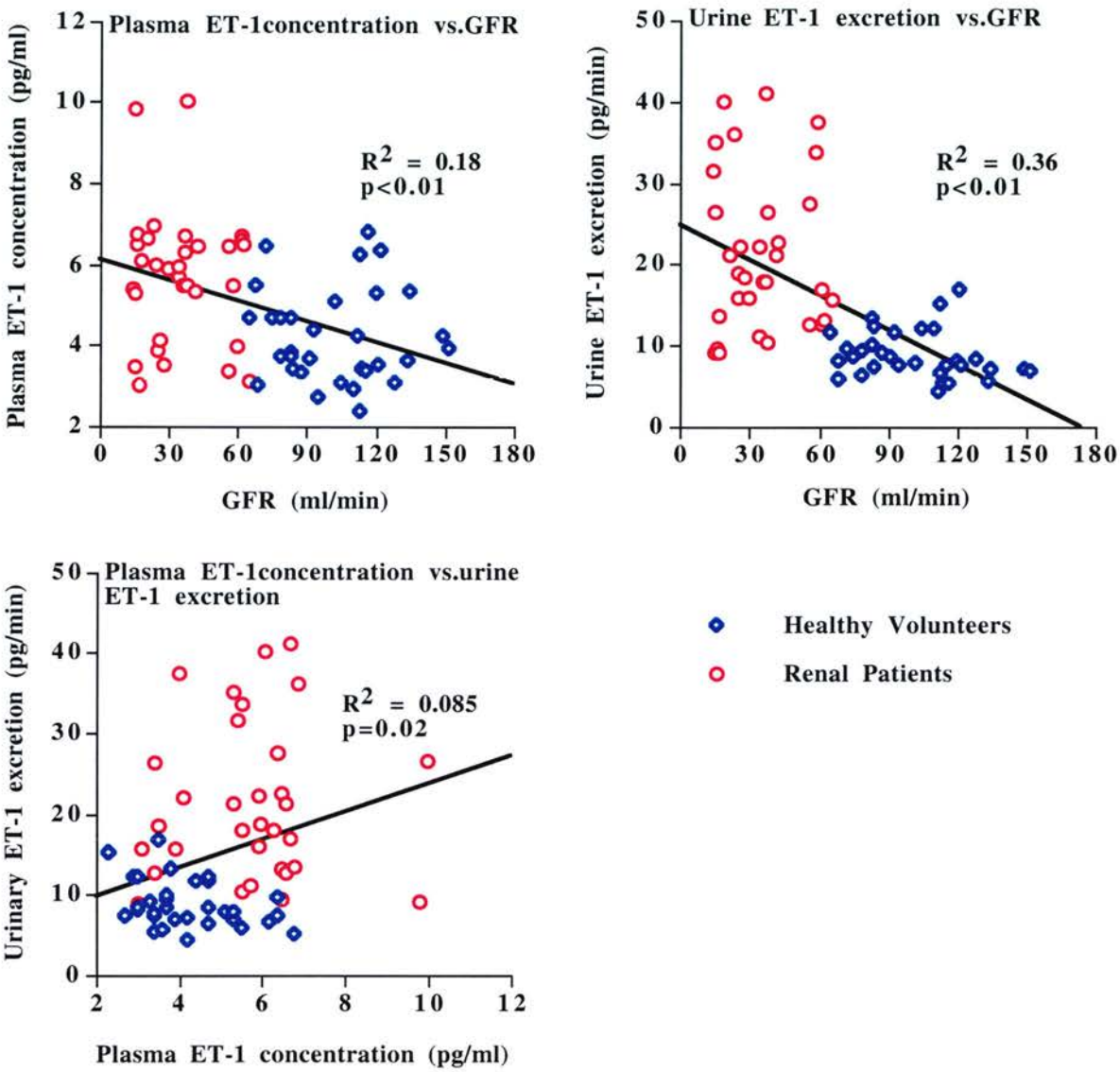


Figure 10.2 Fe ET-1 vs. GFR

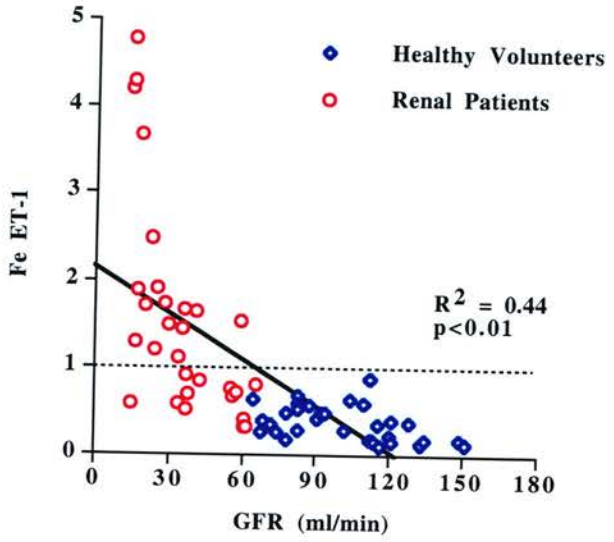
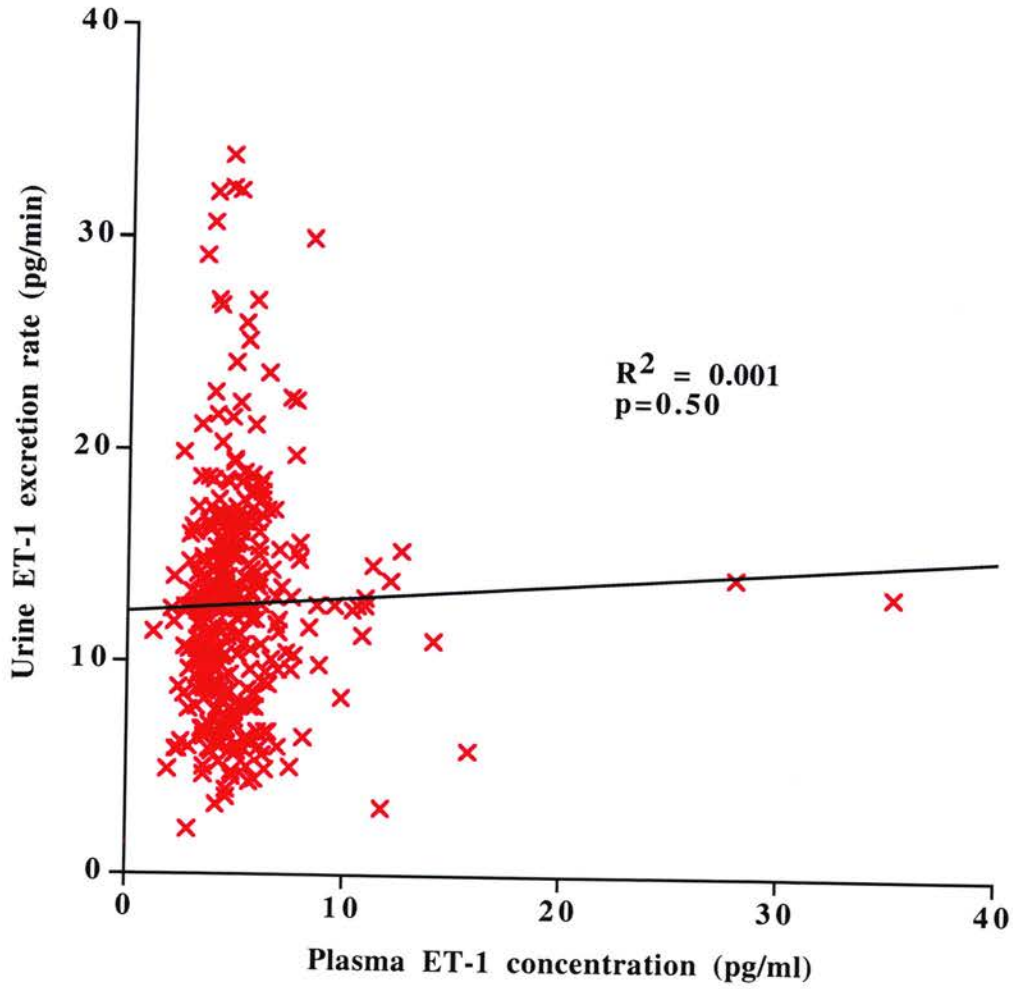
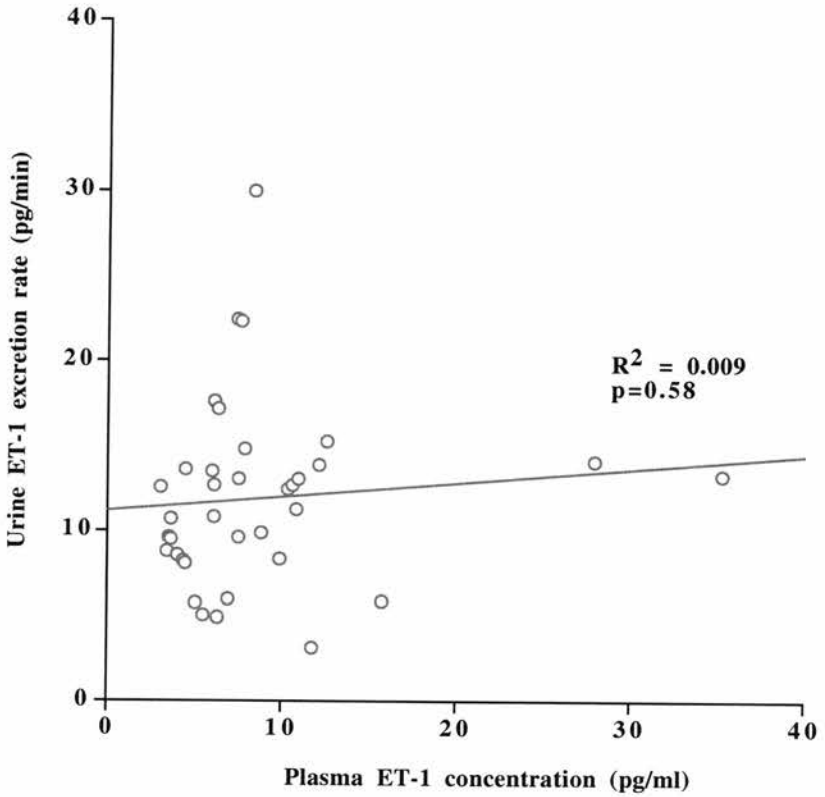


Fig 10.3 Plasma ET-1 concentrations vs. Urinary ET-1 excretion rate - all samples



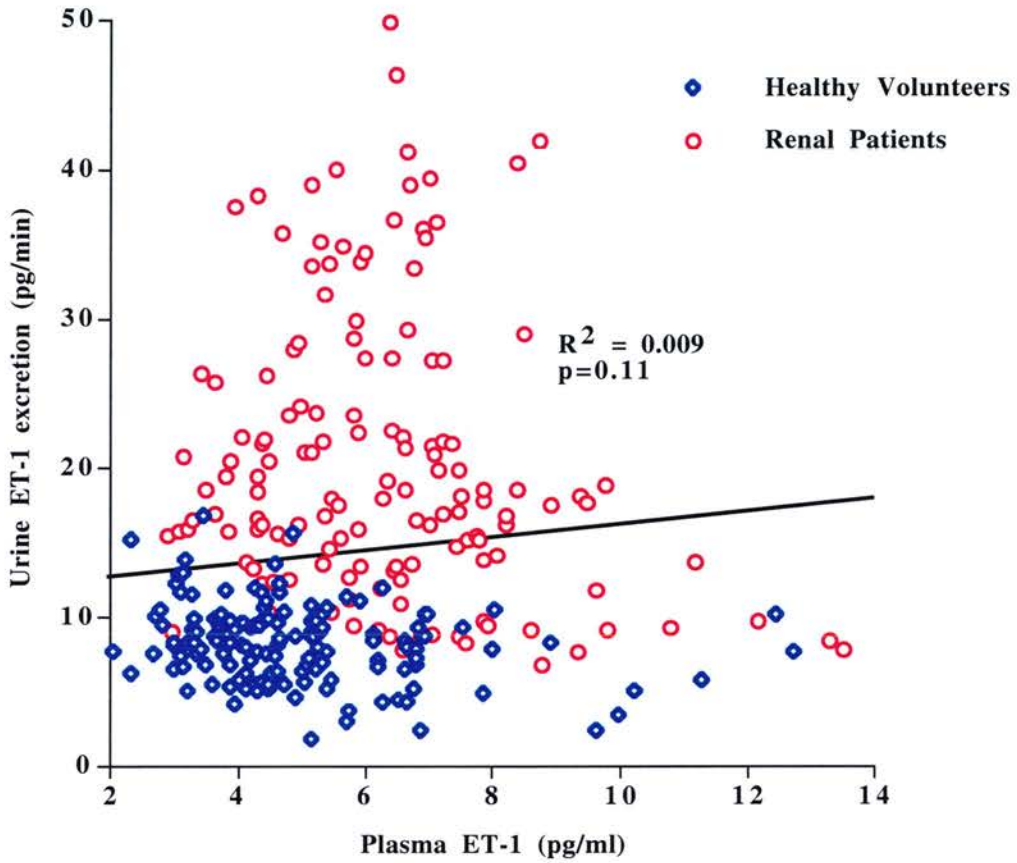
Plasma ET-1 concentrations were significantly increased acutely in two studies. In study 6 (Chapter 8), administration of exogenous ET-1 achieved a ~ threefold increase in plasma ET-1 concentrations but did not alter urinary ET-1 excretion rates (R^2=0.009, p=0.58) (Fig 10.4).

Fig 10.4 Plasma ET-1 concentrations vs. Urinary ET-1 excretion rate during exogenous ET-1 administration



In study 3, plasma ET-1 concentrations increased at 30 min after administration of the high dose of BQ-788 alone (+2.6±0.5, $p<0.01$), or in combination with BQ-123 (+3.5±0.6 pg/ml, $p<0.01$). Again, these alterations in plasma ET-1 after ET receptor antagonism were not reflected by changes in urinary ET-1 excretion ($R^2=0.009$, $p=0.11$) (Fig 10.5).

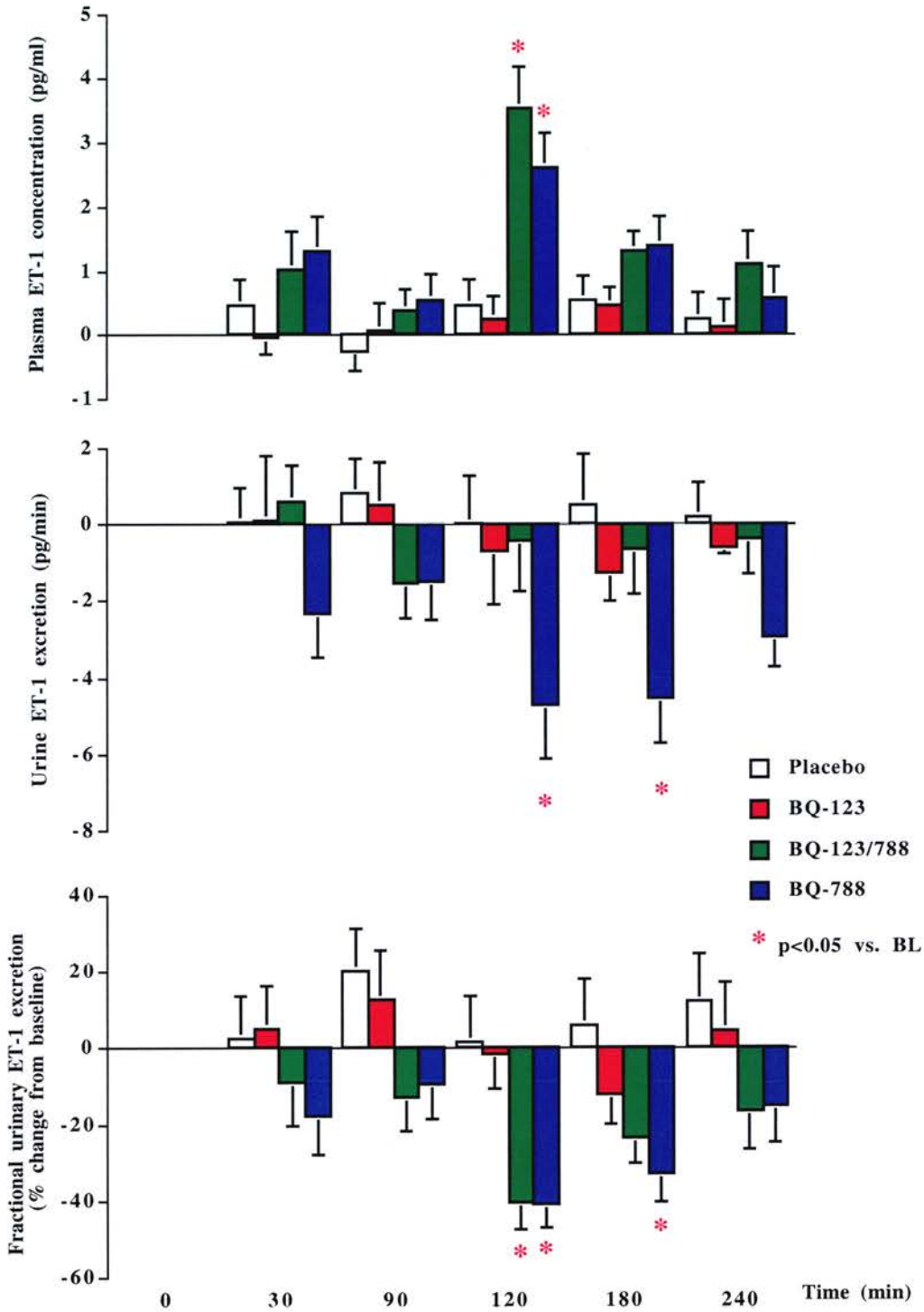
Fig 10.5 Plasma ET-1 concentrations vs. Urinary ET-1 excretion rate during ETB receptor blockade



10.3.2 Effect of ET receptor antagonism on ET-1 excretion

In study 3, following administration of BQ-788 alone urinary ET-1 excretion decreased (-4.7±1.5 pg/min, $p<0.05$) (Fig 10.6) but was not altered by placebo, BQ-123 or BQ-123/788. Because GFR was also reduced by ETB receptor antagonism (-11.4±3.4%, $p<0.01$ vs. placebo), fractional urinary excretion of ET-1 was calculated. This was reduced after both BQ-788 alone (-41±6%, $p<0.01$) and in combination with BQ-123 (-40±7%, $p<0.01$), but not by placebo (+6±12%) or BQ-123 alone (-12±8%) (Fig 10.4). No difference was observed in the pattern of responses to ET receptor antagonism between healthy volunteers and CRF patients. In study 4 (Chapter 6), when healthy volunteers were pre-treated with ACE inhibitor, again a reduction in fractional excretion of ET-1 was observed when BQ-123/788 (-23±13%) but not BQ-123 (-6±18%) was given.

Figure 10.6 Plasma ET-1 concentrations and urinary ET-1 excretion after ET receptor antagonism



10.4 Discussion

The lack of correlation between plasma ET-1 concentrations and urinary ET-1 excretion rates, particularly when drug manoeuvres acutely increase plasma ET-1 concentrations, demonstrates the independence of plasma and urinary ET-1 concentrations, consistent with two separate ET systems in the circulation and the kidney. Additionally, because urinary ET-1 is dramatically and acutely reduced by ETB blockade, these data also suggest a role for ETB receptors in the excretion of ET-1 of renal origin.

Baseline data from study 3 (Chapter 5), before pharmacological manoeuvres to change plasma ET-1, confirm an inverse correlation between plasma ET-1 concentration and GFR, consistent with reduced renal clearance, and between urine ET-1 excretion and GFR, consistent with increased renal production of ET-1 in CRF. Because both plasma ET-1 concentrations and urine ET-1 excretion are related to GFR, a weak correlation does exist between these two measurements at baseline. Additionally, baseline data from study 3 demonstrate that fractional excretion of ET-1 only exceeds unity in patients with CRF. For this to occur more ET-1 must be excreted than filtered. If, as suggested by isotope studies [92], plasma ET-1 does not account for urinary ET-1, a fractional excretion exceeding unity demands renal ET-1 synthesis. Hence our finding supports urinary ET-1 excretion as being a marker of the increased renal ET-1 production in CRF.

There are few data, however, to suggest how renal ET-1 reaches the tubules. A recent study in fish has localised ETB receptors to the basolateral aspect of proximal tubular cells and has demonstrated that low concentrations of ET-1 are able to reduce cell to tubular lumen transport of methotrexate and CyA derivatives. This effect was inhibited by ETB receptor antagonists [437], suggesting a role for ETB receptors in tubular transport mechanisms.

In study 3 and 4, after correcting for changes in GFR by calculating FeET-1, it was evident that urinary ET-1 excretion is reduced specifically by blockade of the ETB receptor either alone or when combined with ETA receptor blockade. Because the acute

nature of our studies make it unlikely that ET receptor blockade had significant effects on renal ET-1 production, these data suggest that the renal tubular excretion of ET-1 appears also to be at least partly mediated through the ETB receptor.

In conclusion, these data provide confirmatory evidence in man for two independent ET systems in the circulation and in the kidney, both related to GFR, and suggest, for the first time, that ETB receptors may be involved in the urinary excretion of renal ET-1. This has implications for the use of ET antagonists that block both the ETA and the ETB receptor. ET-1 is associated with renal fibrosis [438], and therefore failure to excrete renal ET-1 may be detrimental to the kidney, limiting the use of combined ETA/B receptor antagonists. As ET receptor antagonists are in Phase III clinical trials, this important observation warrants further attention.

Chapter 11

Renal Clearance of BQ-123

11.1 Introduction

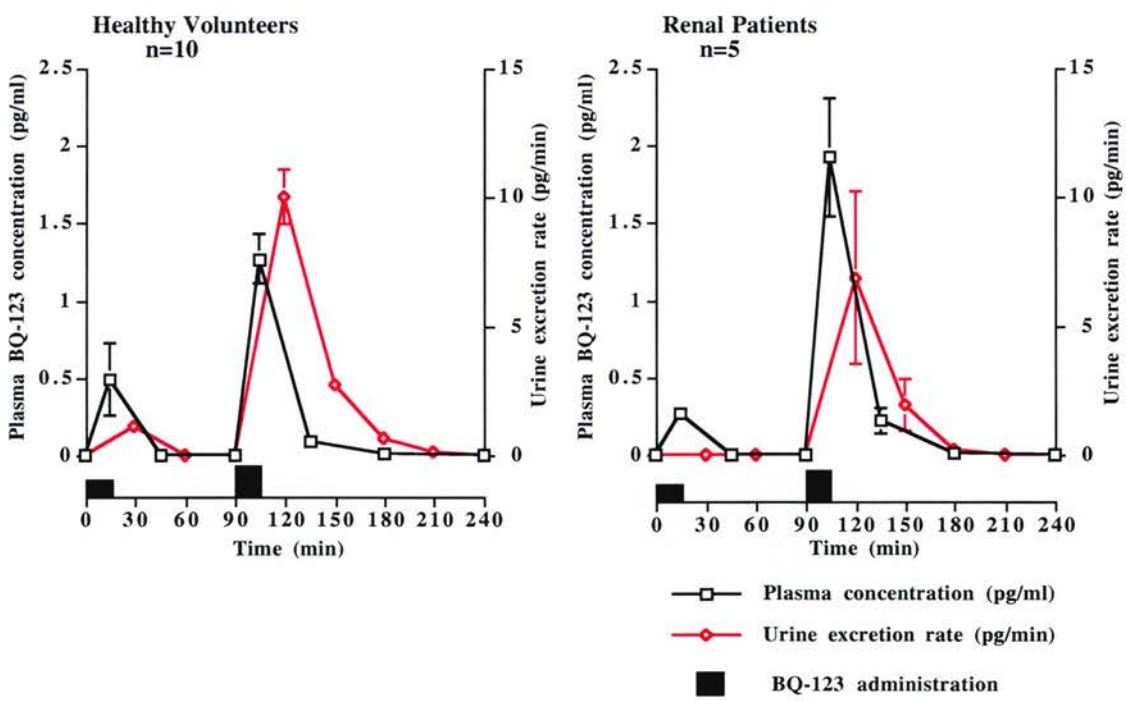
BQ-123 is a cyclic pentapeptide that is cleared by hepatic anion transport mechanisms [97]. However, though non-significant, renal patients had higher plasma concentrations after BQ-123 infusion than healthy volunteers for the same dosing schedule of BQ-123 (study 3). Concentrations of BQ-123 were therefore measured in the urine in a subgroup of subjects to ascertain if any BQ-123 was excreted renally, and, if so, whether this renal clearance was significantly reduced in CRF to account for the higher plasma concentrations observed in CRF patients.

Urinary concentrations of BQ-123 were therefore measured after BQ-123 administration (alone or with BQ-788) during 10 healthy volunteer visits and 5 patient visits from the study described in Chapter 5.

11.2 Results

BQ-123 was recoverable in the urine after intravenous administration. The profile of BQ-123 appearance in the urine mirrored exactly the profile in the plasma (Fig 11.1).

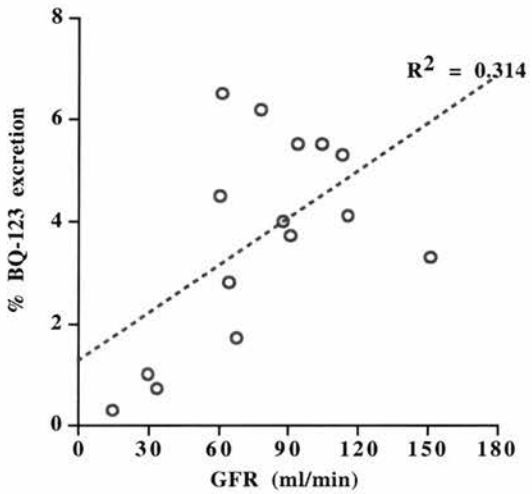
Fig 11.1 BQ-123 plasma concentrations and urinary excretion rates



It fell to zero and remains there by 2 hours after the high dose infusion. In the group as a whole, the total excreted was 3.7 ± 0.5% (0.3 - 6.2%) of the total given.

An inverse correlation between peak plasma concentrations of BQ-123 at 105 min (at end of high dose BQ-123 infusion) and urinary BQ-123 excretion rate between 90 and 120 min was noted (R^2=0.35, p=0.04). Additionally, a correlation between the total BQ-123 excreted by an individual and their GFR was noted (R^2=0.31, p=0.03) (Fig 11.2).

Fig 11.2 % BQ-123 excreted vs. GFR



11.3 Discussion

BQ-123 is subject to renal clearance and this clearance reduces as GFR falls. However, this renal excretion is of minor importance in respect of overall clearance of BQ-123 from the circulation. A reduced urinary excretion rate of BQ-123 is, nevertheless, associated with higher plasma concentrations, and must be taken into consideration in interpreting the effects of BQ-123 in renal patients. Looking at individual plasma concentrations, this is probably only relevant in patients with a very low GFR (<20 ml/min).

Chapter 12

The effects of single high dose cyclosporin in healthy volunteers

12.1 Introduction

CyA is a fungal derived peptide that is a widely used immunosuppressive agent that inhibits the immune system by suppressing T-helper cell activation via inhibition of the intracellular enzyme calcineurin. The introduction of CyA in the transplant field in the 1980s has significantly improved graft survival [439-441]. Unfortunately, though it is an effective immunosuppressive drug, it has a wide range of side effects including hypertension and nephrotoxicity. It is estimated that 60 - 90% of patients on chronic CyA therapy requiring anti-hypertensive medication [441-443].

The precise mechanisms of CyA induced hypertension and nephrotoxicity however, remain unclear. Impaired vasodilator responsiveness [444] or enhanced vasoconstrictor tone, either as a direct CyA effect [445] or as a consequence of increased sympathetic nervous system (SNS) activity [445-447] and renal vasoconstriction, with salt and water retention and consequent extravascular volume expansion [448], have all been proposed, and it is probable that more than one mechanism is acting to increase blood pressure.

Animal models and patient studies have both demonstrated increases in systemic vascular resistance and reductions in CO in response to CyA [449-452]. CyA is also well documented as causing acute renal vasoconstriction in patients on chronic CyA treatment [453-456]. However, while acute studies in healthy volunteers have demonstrated renal hypoperfusion after a single high dose of CyA [347, 457-459], this is not a uniformly reproducible effect [460, 461], and experimental data suggests that, in both healthy subjects and patients, systemic and renal vasoconstriction may only develop after days to weeks of CyA treatment [462-465]. Additionally, forearm studies have suggested that acute CyA administration might, paradoxically, increase the activity of endothelial vasodilators in healthy subjects naïve to the drug [466].

The purpose of this study was, therefore, to examine the systemic and renal haemodynamic effects of a single, oral, high dose of CyA in healthy volunteers to ascertain if the pattern of systemic and renal haemodynamic changes produced differed

from those documented in patients on chronic CyA therapy, and thus ascertain if this is a relevant model for the investigation of chronic CyA induced systemic vasoconstriction and renal hypoperfusion.

12.2 Study design

The study was a randomised, double-blind, placebo-controlled, crossover study. Subjects attended for 2 visits, separated by ≥5 days. Twelve healthy volunteers were recruited to the study. For subject demographic data see Table 12.1

On each day, subjects then underwent an adapted clearance study. After baseline measurements over three 20 min collection periods, subjects then received placebo (olive oil) or CyA (Neoral) liquid (Novartis), 10 mg/kg. Measurements then continued at 20 min intervals for 4 hours. At 0, 60, 120, 180 and 240 min, blood was collected for the measurement of plasma CyA and ET-1.

Table 12.1 Subject demographic data

Age (yr)	33±2 (22 - 44)
Body mass index (kg/m^2)	24 ± 1 (20 - 26)
MAP (mmHg)	90.3 ± 1.8 (80.0 – 99.3)
Creatinine (μmol/L)	84 ± 3 (71 - 104)

12.3 Results

6 subjects completed the CyA arm of the study and 9 completed the placebo arm. The most common reason (in 5 of 24 studies) for non-completion was emesis. Other reasons were not study related. Data was only analysed from those subjects completing a study day. Baseline data did not differ between CyA and placebo phases (Table 12.2).

12.3.1 Systemic haemodynamics

Mean arterial pressure rose in the CyA group by 6.7 ± 1.9% from 92.9 to 99.6 mmHg ($p<0.001$ compared to placebo). This was accompanied by an increase in HR of 26.2 ± 3.5% ($p<0.001$), an increase in CI of 20.6 ± 3.1% ($p<0.001$) and a fall in SVRI of 13.5 ± 1.4% ($p<0.001$). Changes were maximal at 80 min but persisted throughout the 4 hours of the study (Fig 12.1).

Table 12.2: Baseline data

	CyA	Placebo	CyA vs. Placebo *t-test*
MAP (mmHg)	92.9±2.1	89.9±3.0	NS
SVRI (dyne.s m^2/cm^5)	2432±152	2448±232	NS
CI ($L/min/m^2$)	3.1±0.2	2.9±0.2	NS
HR (bpm)	57.3±3.4	54.3±2.6	NS
ERBF (ml/min)	914±90	862±103	NS
ERVR (mmHg.min/L)	116±16	122±17	NS
EFF (%)	27±2	24±3	NS
GFR ($ml/min/1.73m^2$)	120±8	103±7	**NS**
UNaV (μmol/min)	130±41	106±17	NS

12.3.2 Renal haemodynamics

A moderate fall in GFR was seen (-9.0 ± 4.8%, $p<0.01$), maximal at 180 min. ERBF after CyA was not different from placebo. However, ERBF as a proportion of CO did fall from 14.8 to 11.6% ($p<0.01$) (Fig 12.2).

12.3.3 Urinary sodium excretion

There were no differences in urinary sodium excretion between the two groups.

Figure 12.1 Systemic haemodynamics

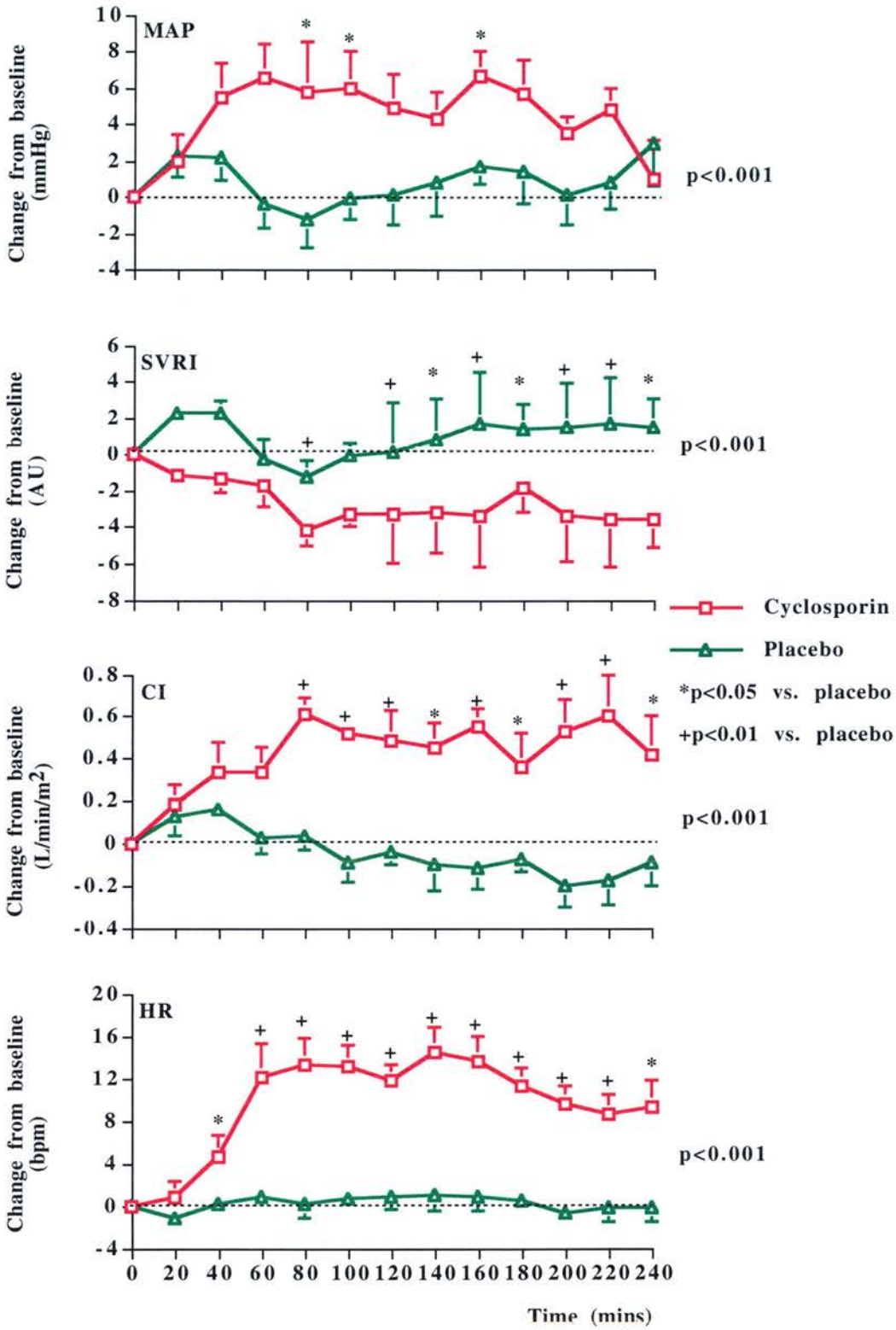
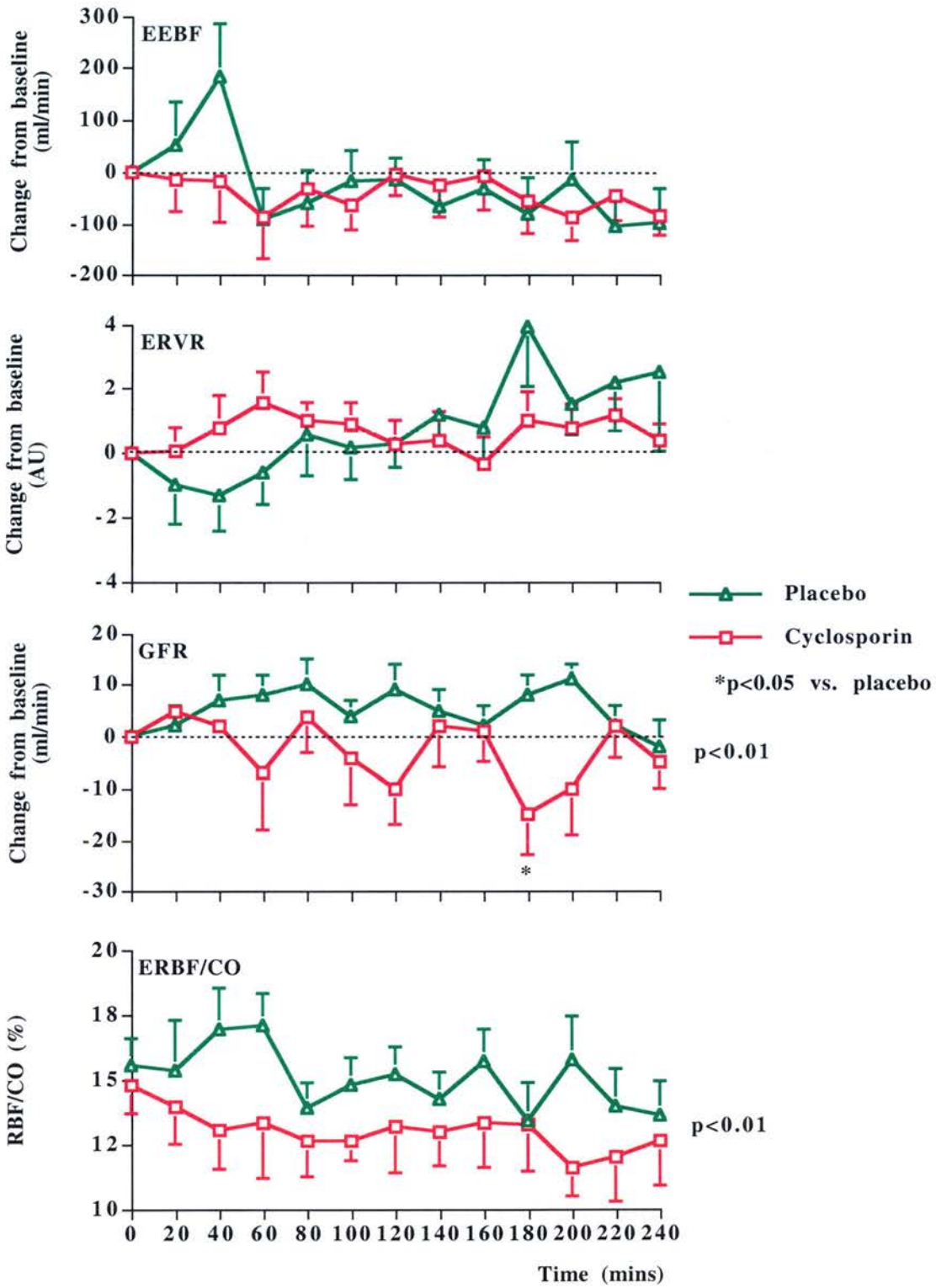


Figure 12.2 Renal haemodynamics



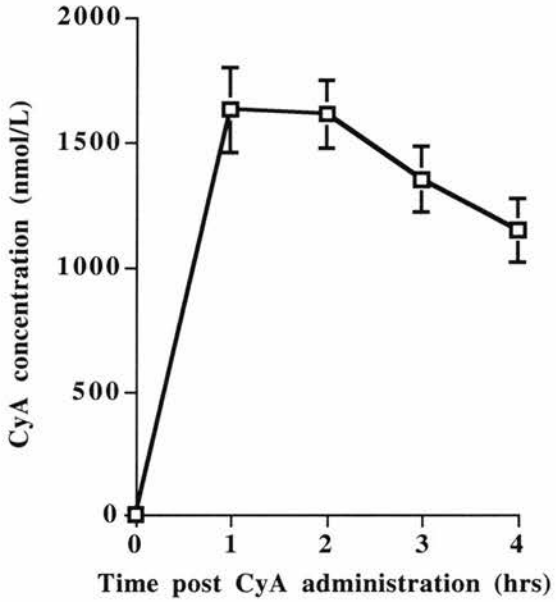
12.3.4 Plasma ET-1 concentrations

Plasma ET-1 concentrations were not altered by CyA administration (Placebo baseline: 3.5 ± 0.9 pg/ml, t=4 hrs 2.7 ± 0.5; CyA baseline 4.8 ± 2.8 t=4 hrs: 3.9 ± 0.6)

12.3.5 Plasma CyA concentrations

CyA concentrations were maximal at 60 minutes post administration and remained at this level for a further 60 minutes reaching peak values of 1630 ± 169 nmol/L (Fig 12.3).

Fig 12.3 Plasma cyclosporin concentrations after administration of 12 mg/Kg oral neoral



12.4 Discussion

Single, high dose CyA in healthy volunteers caused the expected increase in blood pressure, together with in a striking increase in HR. However it was also associated with a decrease in SVRI and an increase in CI. This contrasts with the reported haemodynamics in patients on chronic CyA therapy where an increase in SVRI and a

reduction in CI are commonly seen [451, 452]. Additionally, the renal vasoconstriction reported in patients on treatment [347, 453, 454, 456] was not seen though there was a fall in RBF relative to CO.

Recent forearm studies in healthy volunteers have shown that acute administration of CyA results in increased basal and stimulated NO activity and NOS gene expression [466]. Conversely, renal transplant patients on chronic CyA show reduced basal and stimulated NO activity compared to healthy volunteers [467]. CyA is also known to increase gene transcription and secretion of ET-1 from endothelial and smooth muscle cells [171]. Increased activity of the ET system is also indicated by the ability of ET receptor antagonism to attenuate CyA induced smooth muscle cell contraction [171] and CyA induced renal vasoconstriction [145]. Changes in gene transcription will not be manifest immediately, but will take some days to develop fully. Hence, while CyA acutely upregulates endothelial NO activity, chronic exposure to CyA will cause endothelial dysfunction due to both reduced NO activity and increased ET-1 activity, altering the balance of vasoactive compounds in the endothelium in favour of vasoconstriction. Rat models suggests that this switch in endothelial balance to favour vasoconstriction happens early, with 1-3 weeks being sufficient for reduction in urinary NO metabolites and alterations in vessel responses suggestive of reduced NO activity to occur [468, 469]. Such data suggest the mechanisms behind the hypertensive and renal haemodynamic changes of a single high dose of CyA will be different from those found in chronic CyA administration. The results from this study are consistent with this hypothesis.

The most striking change in this study was the increase in HR after CyA. This study was not designed to look the sympathetic nervous system (SNS), however, it is known that CyA can increase SNS activity [446, 447]. The increase observed in this study was observed in subjects who did not experience nausea after CyA, suggesting this was a direct CyA effect. Such large increases have also been observed after 10mg/kg CyA (Hansen). In the study presented in Chapter 13, subjects given 1-2 mg/kg exhibited small

increases in HR, more consistent with the published literature. This would suggest that the marked increase seen in this study is probably related to the large size of the dose given. Such SNS stimulation will have haemodynamic consequences. As the systemic vasodilatation observed in this study would normally reduce blood pressure, and given that the time course of the study is too short for significant salt and water retention to occur, it is possible that the increase in MAP after CyA in this study is due to this increase in SNS activity. This is provides a second reason why an acute single high dose model of CyA administration might be inappropriate for the investigation of chronic CyA induced changes.

Finally, subjects experienced nausea and vomiting in 5/24 studies and had to discontinue the protocol. Water loading undoubtedly contributed to the nausea as one subject vomited in the placebo phase and before drug dosing. However, 4/5 subjects vomited during the CyA arm of the study with the time course of the vomiting correlating with peak plasma CyA concentrations. This high incidence of emesis renders this model both ethically and physiologically unsound.

In summary therefore, the systemic vasodilatation and lack of changes in RBF in healthy volunteers given single high dose CyA concurs with forearm studies suggesting an initial upregulation of NO in the endothelium in response to CyA. It is at variance with the reported haemodynamic changes in subjects receiving chronic CyA treatment, namely systemic and renal vasoconstriction. This makes an acute single high dose model is an inappropriate one for the assessment potential therapeutic treatments for CyA induced hypertension and nephrotoxicity. The time course of the switch from dilatation to constriction, and its correlation with the induction of endothelial dysfunction by CyA needs more detailed investigation in man.

Chapter 13

The haemodynamic response to cyclosporin in renal transplant patients

13.1 Introduction

Study 8 (chapter 12) demonstrated that in healthy subjects receiving a single large dose of CyA, the acute increase in MAP seen is associated with an increase in CO and a decrease in SVR. In animal models, short-term administration of CyA leads to an increase in SVR and MAP and a decrease in CO [449, 450]. Similar haemodynamic changes have been reported in heart transplant patients when compared with essential hypertensives matched for blood pressure [451]. These different responses to CyA in heart transplant patients compared to healthy subjects might be explained by the altered cardiac responses of a denervated transplanted heart, or the long-term exposure to CyA in these patients. However, vasoconstriction to a single dose of intravenous CyA has also been reported in renal patients on dialysis, but not on chronic CyA treatment [452], suggesting other factors, such as renal impairment, might be involved in CyA induced vasoconstriction.

The purpose of this study was, therefore, to explore the effects of a single standard oral dose of CyA on systemic haemodynamics renal transplant patients to observe if chronicity of CyA treatment was important in the generation of systemic haemodynamic responses and hypertension. To asses this, the systemic haemodynamic responses of renal transplant patients on chronic immunosuppressive therapy including CyA was compared with matched renal transplant patients on long-term immunosuppressive regimes consisting of prednisolone and/or azathioprine (Pred/Aza) but not including CyA. The study hypothesis was that a single dose of CyA would induce vasoconstriction in patients on long-term CyA but not in patients previously naïve to CyA.

13.2 Study design

The study was a randomised, single-blind, placebo-controlled, crossover study in 22 renal transplant patients. Subjects attended for 2 visits, separated by ≥5 days. Subjects continued to take all their normal medications except for CyA, where relevant, which was omitted on the morning of the study only. Subjects received CyA 100 mg on one visit and placebo

(icing sugar) on the other. Both CyA and placebo were placed within gelatin capsules to ensure that the subjects remained blinded to the treatment received on each study day. On each study day, an 18 SWG cannula was sited in the left antecubital vein. Subjects then rested supine for 40 min before study drug administration. Subjects remained recumbent throughout the study. Haemodynamic measurements were made at 10 min intervals from 30 min pre-dose until CyA administration, then at 20 min intervals until 3 hours after dosing. At 0 and 3 hours, blood samples were taken for the measurement of plasma ET-1

13.2.1 Subjects

Eleven renal transplant patients on chronic immunosuppressive therapy including CyA, Pred and Aza (Group I) and eleven patients maintained on immunosuppressive therapy with Pred/Aza but not including CyA (Group II), all under long term follow-up by Edinburgh Renal Unit, were recruited to the study. Patients on immunosuppressive agents other than Pred, Aza or CyA were not recruited to the study. Additionally, patients with known ischaemic heart disease, clinical evidence of heart failure, heart transplantation, diabetes mellitus, or other significant cardiac or vascular history were excluded from recruitment.

Table 13.1 Subject demographic data

	Group I **CyA**	**Group II** **Pred/Aza**	*t-test*
Number	11: 6M 5F	11: 9M 2F	
Age (yr)	51 ± 4	48 ± 3	NS
Body mass index (kg/m^2)	27.5 ± .9	26.9 ± 1.2	NS
MAP (mmHg)	100.6 ± 3.1	92.6 ± 2.6	NS
Creatinine (μmol/L)	137 ± 13	140 ± 34	NS
Duration of transplant (months)	55 ± 8	194 ± 20	$p<0.01$
CyA dose - chronic (mg/dose)	2.64 ± 0.27		
CyA given (mg/kg)	1.34 ± 0.06	1.36 ± 0.07	NS

Groups were matched for creatinine, age and weight. However, mean arterial pressure was slightly higher in group I, 9 of the 11 patients in group I, and 8 of the 11 patients in group II were also on anti-hypertensive medication, and the mean duration of immunosuppressive therapy (= duration of transplant) was significantly shorter for this group compared to the patients on long-term Pred/Aza only (Table 13.1). For subject demographics and patient characteristics, see Tables 13.1 & 2

Table 13.2a Subject characteristics

	Sex	Age (yr)	Renal diagnosis	am CyA dose (mg/kg)	HT?	Anti-HT medication	Time since Tx (months)	Cre (μmol/L)	Other medical conditions
1	M	32	Congenital	2.30	No		28	132	
2	F	52	CPN	1.03	No		103	258	
3	M	61	GN	1.58	Yes	Nifedipine	53	140	COAD
4	F	62	PKD	1.59	Yes	Amlodipine	91	133	OA
5	F	64	HT	0.89	Yes	Doxazosin	36	112	
6	F	66	PKD	0.77	Yes	Metoprolol	44		Gout
7	M	27	Alport's	1.92	Yes	Doxazosin Nifedipine	21	180	
8	M	28	Congenital	1.39	Yes	Atenolol Nifedipine	80	92	
9	M	62	GN	1.18	Yes	Atenolol Diltiazem	64	111	
10	M	62	NK	0.93	Yes	Enalapril Nifedipine	63	133	
11	F	46	HT	1.19	Yes	Diltiazem Doxazosin Nifedipine	28	108	

Table 13.2b Subject characteristics

	Sex	Age (yr)	Renal diagnosis	am CyA dose (mg/kg)	HT?	Anti-HT medication	Time since Tx (months)	Cre (μmol/L)	Other medical conditions
12	M	50	NK		No		195	83	
13	M	51	Congenital		No		215	129	
14	M	67	NK		No		240	89	COAD
15	F	29	Congenital		Yes	Nifedipine	117	108	ROD
16	M	33	PKD		Yes	Atenolol	222	90	OP
17	M	45	GN		Yes	Labetalol	118	141	
18	M	48	GN		Yes	Atenolol	295	91	Psoriasis, OP
19	F	49	GN		Yes	Nifedipine	240	96	
20	M	42	GN		Yes	Doxazosin Enalapril	263	121	OP
21	M	59	GN		Yes	Bisoprolol Nifedipine	117	120	
22	M	51	ADPKD		Yes	Doxazosin Enalapril Labetalol Methyldopa Nifedipine	115	473	

M - male, F - female, CPN - chronic pyelonephritis, GN - glomerulonephritis, HT - hypertension, NK - not known, PKD - polycystic kidney disease, COAD - chronic obstructive airways disease, OA – osteoarthritis, OP - osteoporosis, ROD - renal osteodystrophy, Tx - transplant

13.3 Results

All 22 subjects completed the study. No adverse events were reported. Baseline haemodynamic data did not differ on each of the study phases (Table 13.3).

Table 13.3 Baseline data

	Group I Placebo	Group I CyA	Group II Placebo	Group II CyA
MAP (mmHg)	98.1±3.3	101.2±3.8	93.1±3.7	92.2±2.1
SVRI (dyne.s m^2/cm^5)	2846±347	2996±298	2726±136	2755±167
CI (L/min/m^2)	3.07±0.24	3.05±0.28	2.81±0.21	2.81±0.24
HR (bpm)	61.1±2.8	61.7±2.5	66.6±2.8	66.8±2.8

13.3.1 Systemic haemodynamics

There was a small increase in MAP in both groups after acute CyA administration (maximum placebo-corrected change from baseline, group I: + 7.4 ± 3.3 mmHg, ANOVA $p<0.01$ vs. placebo, group II: +6.7 ± 2.5 mmHg, $p<0.01$). HR was unchanged after CyA administration in both groups relative to baseline values, though increased in group II relative to placebo (group I: +2.3 ± 1.2 bpm, $p>0.5$ vs. placebo, group II: +3.9 ± 2.1 bpm, $p<0.01$) (Fig 13.1).

A significant decrease in CI was observed in group 1 (-0.2 ± 0.11 L/min/m2, $p<0.05$). This contrasted with an increase in CI in group II (+0.27 ± 0.10 L/min/m2, $P<0.05$). SVRI increased in group I both in absolute terms and relative to placebo (+460 ± 128 dyne.sec/cm^5/m^2, $p<0.01$) (Fig 13.1). By contrast, in group II SVRI was unchanged relative to baseline and placebo (-257 ± 137 dyne.sec/cm^5/m^2, $p>0.1$) (Fig 13.1). Both CI and SVRI responses were significantly different ($p<0.01$) when group I was compared with group II (Fig 13.2).

Figure 13.1 Effect of 100 mg CyA or placebo on systemic haemodynamics

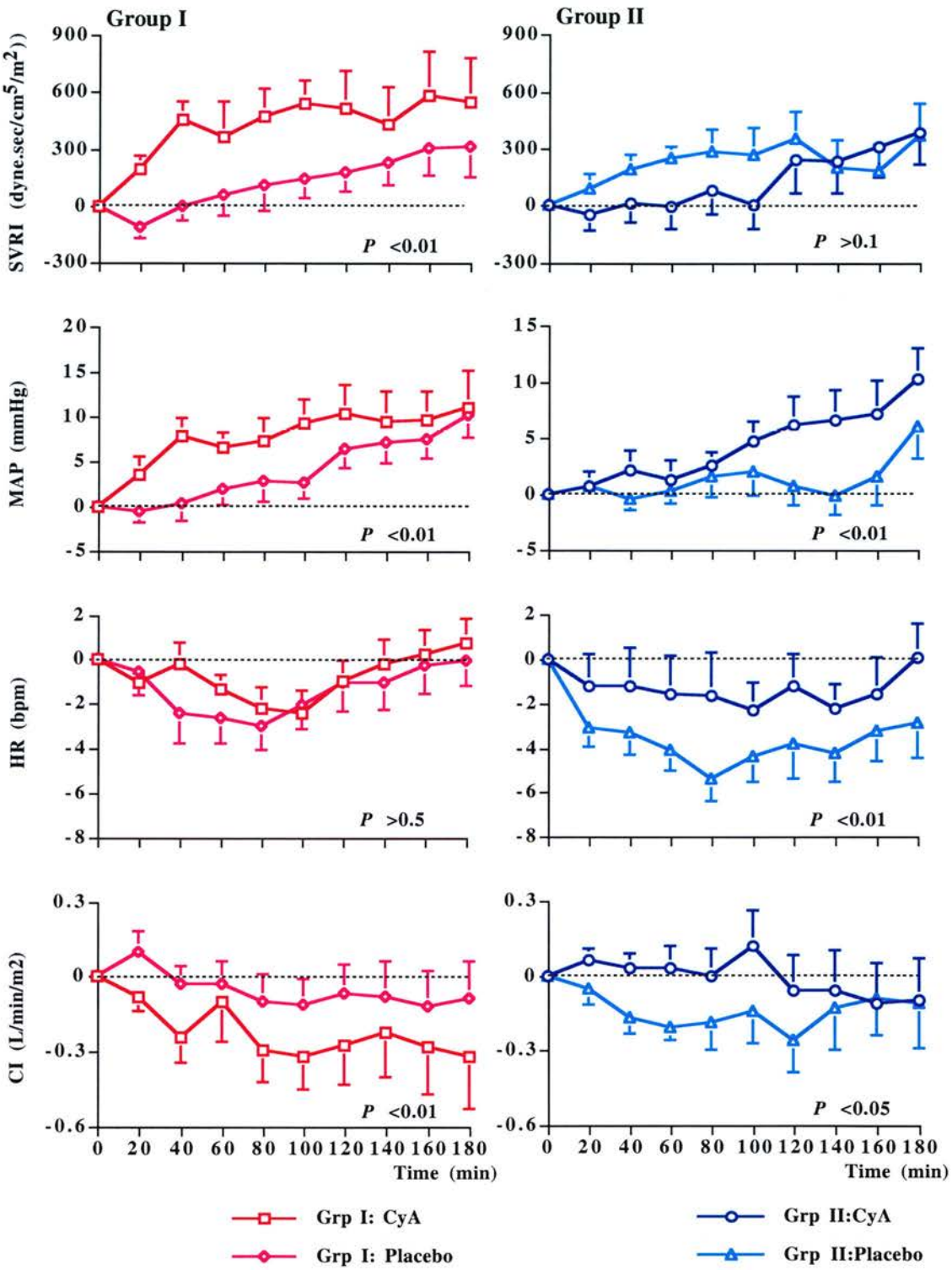
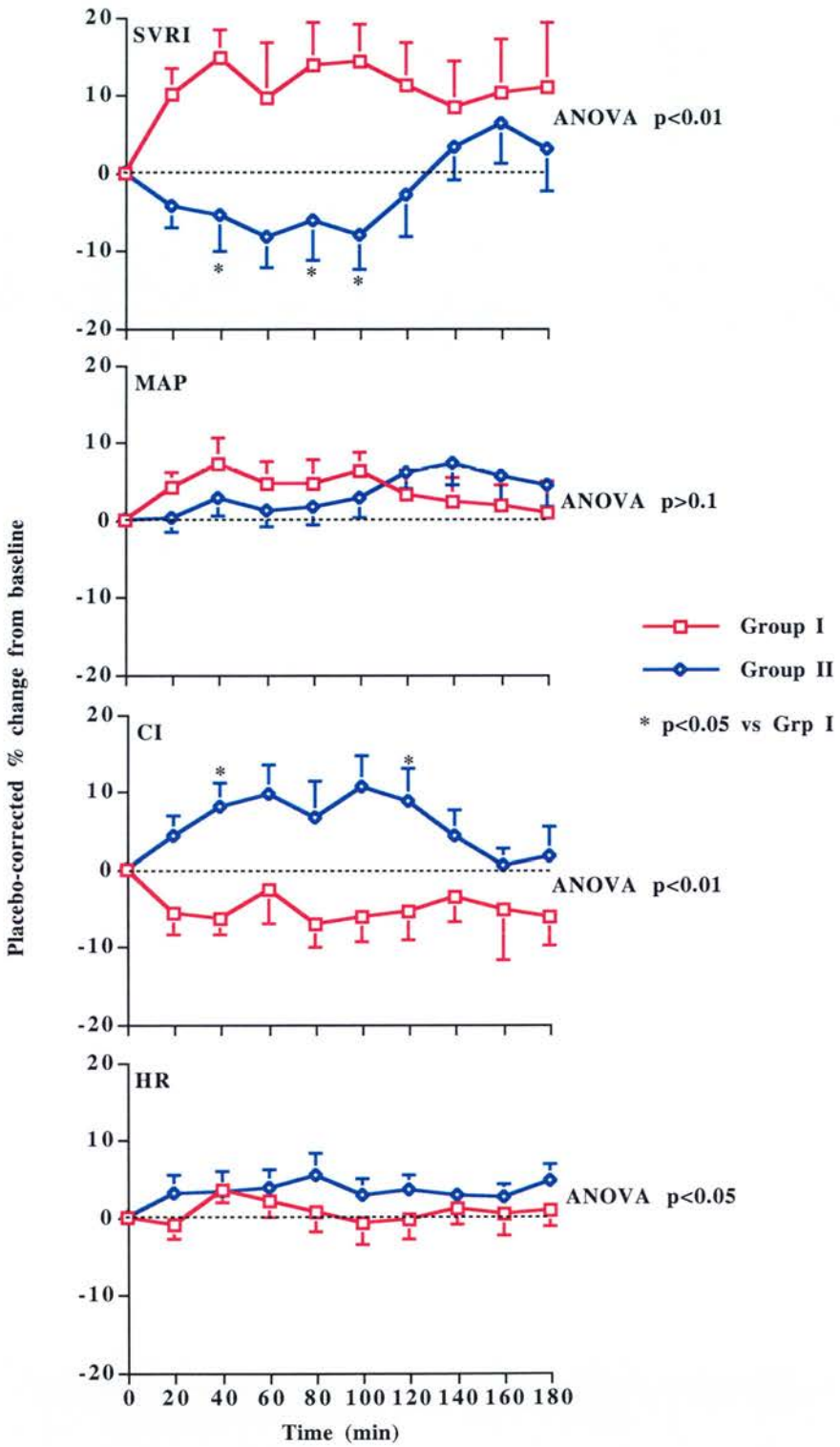


Figure 13.2 Effect of 100 mg CyA or placebo on systemic haemodynamics. Placebo corrected % change from baseline.



13.3.2 Endothelin-1 concentrations

Plasma ET-1 concentrations were not significantly altered in either group compared to placebo after CyA administration (Table 13.4).

Table 13.4 Plasma ET-1 concentrations (pg/ml) after acute CyA administration

	Group I: Patients on long-term CyA		Group II: Patients naïve to CyA	
Study drug	**CyA**	**Placebo**	**CyA**	**Placebo**
Baseline	2.78 ± 0.59	3.85 ± 0.29	5.17 ± 0.21	4.07 ± 0.47
3 hr post administration	4.47 ± 0.41	4.43 ± 0.74	4.32 ± 0.33	4.77 ± 0.16

13.4 Discussion

This study demonstrated that the pattern of the haemodynamic response to acute CyA administration differs between renal transplant patients on chronic CyA therapy and those naïve to it. Patients on chronic immunosuppression with CyA demonstrate an increase in blood pressure associated with an increase in vascular resistance and a reduction in CI after acute CyA administration. By contrast, matched patients maintained on drug regimes without CyA increase both blood pressure and CI and do not vasoconstrict after acute CyA administration. These findings in transplant patients on long-term CyA are consistent with published literature in heart transplant patients and chronic dialysis patients [451, 452]. The patients naïve to CyA, however, followed the pattern previously observed in healthy subjects given a large single dose of CyA (Chapter 12) with a small vasodilatation and an increase in CI.

CyA can induce smooth muscle cell contraction [171], and cause increased contractile responsiveness of blood vessels in vitro [445]. However, in healthy subjects with

presumed normal endothelial function, acute administration of CyA into the brachial artery only causes vasoconstriction in the presence of a nitric oxide clamp, and, in the presence of stimulated endothelial NO release, in fact, increased forearm blood flow, suggesting that nitric oxide is an important regulating mechanism protecting against CyA-associated vasoconstriction in vivo [466]. Morris *et al.* have demonstrated that renal transplant patients on chronic CyA have reduced basal and stimulated NO activity compared to those on Pred/Aza and to healthy volunteers [467]. Hence, chronic exposure to CyA is associated with reduced NO activity, and thus a loss of protection against further CyA associated vasoconstriction. Rat models suggests that this switch in the balance of endothelial vasomotor tone to favour vasoconstriction during CyA administration happens early, with 1-3 weeks being sufficient to see a reduction in urinary NO metabolites and alterations in vessel responses, consistent with reduced NO activity [468, 469].

The results from this study would be consistent with this loss of NO activity in long-term CyA administration. The observed vasoconstriction after acute CyA suggests that chronic exposure to CyA has resulted in endothelial dysfunction with reduced NO activity, and loss of an NO related protective mechanism. The lack of vasoconstriction to acute CyA in the Pred/Aza patients suggests that endothelial function in this group is comparatively well preserved and thus NO activity is protecting against CyA induced increases in vascular tone.

It is possible that, in addition to reduced NO activity during chronic CyA treatment, increased activity of vasoconstrictor systems also occurs. In respect of ET-1, CyA is known to stimulate ET release from endothelial and smooth muscle cells and activates ET-1 gene expression [171]. ET receptor antagonism attenuates CyA induced renal vasoconstriction [145] and CyA induced smooth muscle cell contraction [171]. If ET-1 is upregulated in patients on chronic CyA treatment, and further augmented cyclically by twice daily CyA ingestion, then ET receptor antagonists, which improve endothelial function, might provide a therapeutic option for CyA induced hypertension and renal

dysfunction. Indeed, Binet *et al.* have recently demonstrated that the ET receptor antagonist bosentan can attenuate CyA associated renal vasoconstriction, though not hypertension in healthy subjects treated with 7 days of CyA [177]. This study does not show any convincing alterations either in baseline plasma ET-1 concentrations between the two groups studied, or after single dose CyA within each group. However the secretion of ET-1 from endothelial cells is mainly abluminal and plasma concentrations may not be truly reflective of alterations in the activity of the ET system. Further studies in patients with ET receptor antagonists are needed to clarify this issue.

It must be noted, however, that, despite differences in SVRI and CI, similar rises in MAP occurred in both groups, thus blood pressure changes cannot be wholly attributed to alterations in vessel tone. Increased SNS activity [446, 447], and salt and water retention [448] have also been proposed as mediators of CyA associated hypertension. This study was not designed to look at either of these possibilities. However, in respect of salt and eater retention, it is highly unlikely that, given the short time course, this can account for the acute blood pressure response seen here. Previous studies have suggested that hypertension precedes the onset of antinatriuresis by some hours [347] to days [461, 464, 465] supporting the hypothesis that hypertension on CyA is unlikely to be initiated by renal vasoconstriction and consequent sodium retention.

The groups studied were well matched except for the length of time that they had been transplanted. It is important to note, given the association of renal impairment with endothelial dysfunction [470], and the finding that acute administration of CyA to dialysis patients naïve to CyA causes vasoconstriction [452], that they have similar creatinines. The different responses between the groups to CyA observed in this study are therefore not associated with differences in renal function. The two groups do differ, however, in the duration of immunosuppressive treatment. Since the introduction of CyA in the early 1980s, dual therapy with Pred/Aza has become rare, with most immunosuppressive regimens containing a calcineurin inhibitor. As such, the Pred/Aza treated patients represent an historical group. Hence it is not possible to match for

duration of transplant with CyA treated patients. However, one would anticipate greater not less endothelial dysfunction with increasing time as a renal patient.

In summary, the observed haemodynamic response of renal transplant patients to CyA is altered by previous long-term exposure to CyA from mild vasodilatation to vasoconstriction, consistent with CyA associated endothelial dysfunction. Identification of such a chronic CyA induced endothelial dysfunction is important, as restoration of endothelial balance in favour of vasodilatation might help protect against both hypertension and the renal vasoconstriction common in CyA treated patients. Further work is needed to clarify the time course over which this endothelial dysfunction develops in man, and its relative contribution to the overall elevations in blood pressure seen in CyA treated patients.

Chapter 14

Conclusions

The acute studies presented in this thesis explore the acute actions and interactions of ET-1 and its receptors in the systemic and renal circulations in health and disease. These studies have helped to establish a clearer picture of ET physiology and pathophysiology. Such observations suggest there is clinical potential for ET receptor antagonists in the treatment of renal dysfunction. As indicated in Chapter 1, *in vitro* and *in vivo* observations suggest a pathogenic role for ET-1 and a possible therapeutic role for ET receptor blockade in a number of cardiovascular and renal diseases. In this respect, the observations in this thesis in respect of ETA receptor antagonism, particularly in conjunction with ACE inhibition are promising. However, the observations of these acute studies need to be borne out by long-term clinical intervention studies.

14.1 Role of ET-1 and ETA in the maintenance of normal vascular tone

These studies suggest a role for ET-1 acting via the ETA receptor in the systemic but not renal vascular tone in health.

Though initial studies with ET-1 and ECE inhibitors or ET receptor antagonists suggested a role for ET-1 in the maintenance of normal vascular tone [191, 192, 194, 195, 201], further local [197, 471] and systemic studies [139, 142, 199, 202] with ET receptor antagonists have been conflicting in respect of this function of ET-1.

The studies in this thesis in healthy subjects repeatedly demonstrate systemic vasodilatation with ETA receptor antagonism when this is used at doses sufficient to produce significant ETA receptor blockade, consistent with a role for ET-1 acting via the ETA receptor in the maintenance of normal vascular tone. However, consistent with previous studies, these data suggests that ET-1 does not play a role in the maintenance of renovascular tone in the "unchallenged' renal circulation in health [120, 139-141, 199].

It is important to remember that ETA receptor antagonism can cause vasodilatation not only by blocking ETA mediated vasoconstriction but also by allowing endogenous ET-1 to bind preferentially to the unblocked endothelial ETB receptor promoting ETB

receptor mediated vasodilatation. However, concomitant ETB blockade in healthy volunteers does not alter the systemic haemodynamic effects of ETA receptor antagonism suggesting that it is blockade of ETA mediated vasoconstriction rather than promotion of ETB mediated vasodilatation that is responsible for the major part of the decrease in vascular tone observed after ETA receptor antagonism.

14.2 Role of endothelial ETB receptor mediated vasodilatation and vascular smooth muscle ETB receptor mediated vasoconstriction in the maintenance of normal vascular tone

These studies suggest that the net effect of ETB receptor activation in health is to produce vasodilatation, evident in both systemic and renal circulations.

Local studies with ET receptor agonists and antagonists [207, 208] have suggested that ETB receptors also play a vasoconstrictor role in the maintenance of normal vascular tone. However, there may be a difference depending upon vessel type in respect of the presence and clinical significance of vasoconstrictor ETB receptors.

Local forearm [194] and systemic [210] administration of ETB receptor antagonist in this department has been shown to produce vasoconstriction. The studies in this thesis have confirmed this finding in healthy subjects, suggesting that endothelial vasodilator ETB receptors have a role in maintaining a basal vasodilatation in the systemic circulation, and that the balance of the constrictor and dilator functions of ETB receptors is, in these circulations at least, in favour of vasodilatation in health.

As with ETA receptor antagonism however, ETB receptor blockade may produce vasoconstriction not only by loss of ETB mediated vasodilatation but also by disproportionate binding of endogenous ET-1 to the unblocked ETA receptor producing ETA mediated vasoconstriction. Additionally, loss of the clearance function of the ETB

receptor will increase circulating levels of ET-1 that may further augment ETA receptor activation.

In this respect it is important to note that, contrary to expectations if the clinical effect of ETB receptor activation is vasodilatation, concomitant administration of ETB to ETA receptor antagonism does not affect the systemic vasodilatation produced by ETA receptor antagonism alone. This is in contrast to previous forearm studies when ETB receptor antagonism attenuated the vasodilatation produced by ETA receptor blockade [194]. There are a number of possible reasons for this discordance. Firstly, the forearm circulation is not necessarily indicative of total peripheral vascular resistance. The measurement of SVR may include local circulations where ETB mediated vasoconstriction is important. Secondly, the vasodilatation produced by ETA receptor antagonism may produce reflex neurohormonal mechanisms that act to limit any changes in the circulation. In forearm studies, this is avoided by using concentrations of drugs that are only locally active and so should not activate systemic compensatory mechanisms [365]. Thirdly, the role of ETB mediated vasodilatation in health may be minor by comparison to ETA mediated vasoconstriction and so loss of this vasodilatation in healthy blood vessels in an "unchallenged" state may be clinically insignificant in the presence of blockade of the constrictor ETA receptor. Therefore, the vasoconstriction produced by ETB receptor antagonism alone, and the failure of ETB receptor blockade to augment ETA receptor mediated vasodilatation does not support a role for constrictor ETB receptors in health, but is consistent with a role for ETB in the maintenance of systemic vasodilator tone.

Within the renal circulation, the lack of effect of ETA receptor blockade suggests that ETA mediated tone in not important in the kidney in health. The absence of dilatation after ETA receptor blockade would also suggest that ETB mediated vasodilatation (via binding of "displaced" ET-1) is not important. However, in health, the "unchallenged" renal circulation is near-maximally dilated hence further reductions in renal vascular resistance are unlikely. The renal vasoconstriction seen after ETB receptor blockade

suggests that the endothelial ETB receptor is an important mediator of this dilatation. The fact that concomitant ETA blockade abolishes this dilatation suggests that the ETA receptor does have a constrictor function in the renal circulation that may become more important in circumstances where the renal circulation is "challenged". As with the systemic circulation, the data from this thesis do not support clinically relevant ETB mediated vasoconstriction in the renal circulation in health.

14.3 Role of ET-1 and ETA in the maintenance of vascular tone in renal failure

These studies suggest a role for ET-1 acting via the ETA receptor in the systemic *and*, in contrast to health, in renal vascular tone in patients with CRF.

In respect of systemic haemodynamics, in forearm studies in patients with renal failure, ETA receptor antagonism produced vasodilatation, but to a lesser degree than that seen in healthy controls [195]. Similarly other studies in hypertensives [222] and patients with heart failure [285] did not show enhanced vasodilatation to ETA receptor blockade. Studies where patient groups do show enhanced vasodilatation after ETA receptor blockade compared to controls [221] have used doses of ET antagonists (BQ-123: 100 nmol/min) that are, on the basis of these studies, systemically active, and therefore will activate systemic homeostatic mechanisms that will make interpretation of results difficult. Additionally, it is possible that, locally, this dose reaches sufficiently high concentrations to lose ETA selectivity and begin to have effects at the ETB receptor [198].

However, these systemic studies show an equal effect of ETA receptor antagonism on SVR, and a greater effect on blood pressure in CRF compared to healthy controls. Additionally, renal haemodynamic changes occur only in subjects with renal impairment. In attributing these findings to ETA receptor blockade alone, the preliminary dose ranging study and measurement of BQ-123 concentrations allows a

degree of certainty in stating that maximal ETA receptor blockade has been achieved without losing ETA selectivity. BQ-123 is not therefore directly activating the ETB receptor in these studies.

Therefore, the presence of renal failure and (treated) hypertension may alter the vascular sensitivity to ET receptor antagonists and reflect an enhanced activity of the ET system in renal disease, particularly in the renal circulation. The observed increased urinary ET-1 excretion rates in renal failure would support this argument at least in respect of the kidney. The findings may not be consistent with local studies for the aforementioned reasons pertaining to the extrapolation of local to systemic data. However, there are a number of other possible explanations for these findings. Firstly, ET receptor antagonists may be having direct effects on cardiac function [212] and venous capacitance [472] that may alter blood pressure. An increase in ETA mediated venodilation, or an ETA mediated decrease in CO for example, may result in a greater reduction in blood pressure. These studies were not designed to look at these possibilities beyond a basic measure of CI and HR. Secondly, there is the possibility of altered drug pharmacokinetics in renal failure. BQ-123 is primarily metabolised in the liver [97]. Though these studies have demonstrated some renal excretion of BQ-123 that is GFR dependent, this does not impact significantly on systemic pharmacokinetics and is unlikely to be responsible for the observed differences between patients and healthy subjects. Thirdly, there is the possibility of interactions with other drugs taken by the patients. The studies presented in this thesis investigating the interaction of ET receptor antagonists and ACE inhibition do raise the possibility that the different responses to ET receptor antagonists seen between CRF patients and healthy controls, both in the degree of systemic haemodynamic change and the presence of renal haemodynamic changes, are all attributable to concurrent ACE inhibition in the renal patients, though it is worth noting, that 2 patients that were not on ACE inhibitors had similar responses after ETA receptor antagonism to the other patients.

14.4 Role of endothelial ETB receptor mediated vasodilatation and vascular smooth muscle ETB receptor mediated vasoconstriction in the maintenance of vascular tone in renal failure

These studies suggest that the net effect of ETB receptor activation in renal failure is to produce vasodilatation, evident in both systemic and renal circulations.

Forearm studies in subjects with hypertension [196] or atherosclerosis [473] have demonstrated that ETB receptor antagonism produces vasodilatation, and augments ETA mediated vasodilatation, suggesting an increase in the importance of ETB mediated vasoconstriction in hypertension. It is likely, however, these findings are due to a dose of ETB receptor antagonism (BQ-788: 50 nmol/min) that has been shown to be systemically active in these studies.

The studies in patients with renal impairment do not suggest any increase in the importance of vasoconstrictor ETB receptors in the systemic or renal circulations. By contrast, the ETB receptor appears to be of greater importance in the maintenance of systemic and renal vasodilator tone in renal failure. Not only does ETB receptor blockade alone produce vasoconstriction, but concomitant administration of ETB receptor blockade, does, in renal patients attenuate the haemodynamic effects of ETA receptor blockade, suggesting that ETB mediated vasodilatation is clinically significant in renal disease, particularly in the renal circulation. It is worth noting that absolute renal vascular resistance at baseline in the renal patients is three times that of the healthy volunteers indicating a basal state of renal vasoconstriction, and that the absolute increase in renal vascular resistance, though equal in percentage terms, is also three times that of healthy controls. This suggests that the balance of dilators and constrictors in the blood vessels in CRF is shifted towards constrictors. Thus blockade of one of these constrictors, ET-1 via the ETA receptor, or loss of one of the dilator functions, ET-1 via the ETB receptor, becomes clinically more significant.

Of course, given the demonstrated synergism between ETA receptor antagonists and ACE inhibitors that is abolished by concomitant ETB blockade in healthy volunteers, it is possible that the differential effect of ETA and ETA/B observed in the renal patients but not the healthy subjects is attributable to pre-treatment with ACE inhibition. Again, however, the 2 patients that were not on ACE inhibitors had similar responses to combined ETA/B receptor blockade to the other patients.

14.5 Renoprotective profile of ETA receptor antagonism in renal failure

These studies suggest that ETA receptor antagonism offers a potentially renoprotective profile.

These studies have demonstrated that, acutely ETA receptor antagonism produces systemic vasodilatation and a clinically relevant reduction in blood pressure in patients with renal failure, without sodium retention. This effect, though slightly greater with ETA receptor antagonism, can be seen with combined ETA/B receptor blockade. ET receptor antagonists have been shown in large scale studies to be effective in lowering blood pressure [224], but, given the large numbers of anti-hypertensive agents already available, this alone is insufficient to focus attention on ET receptor antagonists.

However, these studies have also demonstrated that ETA receptor antagonism can reduce RVR and increase RBF in CRF patients. This renal vasodilatation is associated with a reduction in EFF. In the absence of changes in mesangial contraction, this is suggestive of a preferential efferent arteriolar dilatation that will therefore act to reduce glomerular capillary perfusion pressure. Consistent with this proposed reduction in glomerular capillary pressure, there is a reduction in proteinuria.

In support of these observations, a previous study in type I diabetics with proteinuria but essentially normal or only mildly impaired renal function has demonstrated that 6 weeks of ETA receptor blockade does reduce blood pressure and, though not significantly affecting overall renal haemodynamics, does reduce proteinuria [190].

In respect of clinical potential, it is important to note that these renal effects are not produced by combined ETA/B receptor antagonism.

14.6 Natriuresis

These studies have not clearly demonstrated an ET mediated natriuresis.

Animal evidence to date points to a paracrine action of renal ET-1 via the ETB receptor in salt and water homeostasis [135, 394]. The peritubular distribution of ETB receptors [11, 52] suggests that the ETB receptor also mediates these actions in man. These studies have demonstrated that selective ETB receptor blockade does reduce sodium excretion in both health and disease. However, correcting for GFR suggests that his anti-natriuresis is accounted for by renal vasoconstriction and not a blockade of ETB mediated natriuresis. Also, ETA blockade alone, though not producing sodium retention despite a fall in blood pressure, did not increase sodium excretion despite leaving the ETB receptor available to endogenous ET-1. These results are consistent with previous studies in man where no clear effect can be demonstrated.

In administering exogenous ET-1 in the presence of ETA blockade, it was hoped to stimulate an unblocked ETB receptor to observe any tubular effects, an approach used effectively in dogs [123]. However, as discussed, the dose of ET-1 was probably too high for the dose of BQ-123 used, resulting in drug displacement from the ETA receptor, as evidenced by loss of ETA mediated vasodilatation. Thus the failure to demonstrate natriuresis is not indicative of a lack of ETB tubular effects in man.

Only when ETA receptor antagonism was given in the presence of ACE inhibition was a significant, and indeed striking, increase in sodium excretion observed. It is possible that this is entirely a consequence of the renal vasodilatation observed, and therefore again a haemodynamic effect. However, the renal haemodynamic effects are not as great as those seen in renal patients, where no net changes in sodium excretion were observed.

Animal studies suggest that the effects of exogenous ET-1 are regional within the kidney with cortical vasoconstriction and medullary vasodilatation [474]. It is possible therefore that these regional variations are further enhanced with concomitant ETA-ACEI treatment increasing blood flow to the medullary tubular segments promoting sodium excretion. Because concomitant ETB receptor blockade equally abolished the renal haemodynamic and natriuretic changes of this combination treatment, it is impossible for us to attribute this natriuresis directly to the unblocked tubular ETB receptor

14.7 Angiotensin - endothelin interaction

These studies have demonstrated that the acute effects of ANG II are not mediated through ET-1 via the ETA receptor.

Clinically, this is consistent with studies demonstrating an effect of ET receptor antagonism in patients already treated with drugs blocking angiotensin activity [292, 293, 299]. The distinct nature of ANG II and ET-1, at least acutely, therefore allows for ET receptor antagonists to be seen as an adjunctive treatment to RAAS inhibition. However, animal studies suggest a more chronic interaction, particularly in respect of vascular remodelling [371], that needs to be explored in man.

However, a synergistic interaction is demonstrable between ETA receptor blockade and ACE inhibition. As shown, this is largely mediated, through an unblocked ETB receptor, by nitric oxide but not prostaglandin. This synergism may, in part, be responsible for the greater effect of ETA receptor antagonism in renal patients compared to healthy controls. Another possibility, however, is a pharmacokinetic interaction between BQ-123 and enalapril. Though BQ-123 plasma concentrations were not significantly different in the presence or absence of enalapril, they were higher during combination treatment. Given the small numbers of subjects studied, it is possible that a pharmacokinetic interaction has been missed.

These current studies cannot differentiate if the role of ACE inhibition in this interaction is to reduce ANG II formation or reduce the breakdown of bradykinin. However, a synergistic interaction has been demonstrated between angiotensin receptor antagonists and ETA receptor antagonists [410] suggesting the former is more likely.

14.8 Evidence for the deleterious effect of ETB receptor blockade

These studies suggest that all the potentially beneficial haemodynamic effects of ET receptor antagonism are mediated through the ETA receptor. All the data in this thesis points to the net balance between endothelial and vascular smooth muscle ETB receptors being in favour of vasodilatation. Concomitant ETB blockade, at best, does not alter the effects of ETA receptor blockade. In these studies, it reduces the hypotensive and renal vasodilatory effects of ETA blockade in patients with renal failure. Possibly connected to these responses, it also abolishes the synergism that operates between ETA receptor blockade and ACE inhibition.

Additionally ETB is known to act as a clearance receptor for ET-1 [87]. On every occasion where the ETB receptor was blocked in these studies, plasma ET-1 concentrations increased consistent with this clearance role. Though the clinical significance of increased plasma concentrations of this paracrine hormone is unclear, there is some evidence that circulating ET-1 may play an important role in renal regulation in cardiorenal states of ET activation [109].

Finally, ETB receptor blockade reduced the urinary excretion of ET-1. Given the mitogenic potential of ET-1 [64, 65] and the association of increased intra-renal production of ET-1 in low renal mass models correlating with glomerulosclerosis [148, 180-182], the retention of ET-1 within the kidney thus has the potential to be deleterious.

14.9 Studies with cyclosporin

The initial aim of high dose CyA administration to healthy subjects was to provide a model of systemic and renal vasoconstriction for interventional studies with ET receptor antagonists. Instead these studies demonstrated that the initial effect of CyA is to produce vasodilatation, both in healthy subjects, and in transplant patients naïve to the drug. This is consistent with forearm studies in transplant patients where patients on CyA demonstrate a greater degree of endothelial dysfunction than matched controls [467], and in healthy subjects demonstrating that CyA acutely increases NOS activity on first exposure [466].

14.10 Future Studies

The observations in this thesis raise further questions to be answered and areas to explore. Some of these are discussed below

1. Clarification of the effect of ETA receptor antagonism on proteinuria

The reduction in proteinuria in renal patients after ETA receptor antagonists was clearest in subjects with higher baseline degrees of proteinuria. The renal subjects recruited had low grades of proteinuria (all < 1.5 g/24hr). Nephrotic range proteinuria was a specific exclusion criteria to avoid issues with altered protein binding of drugs in the circulation. Given that evidence with ACE inhibitors is that the greater renoprotective effect is afforded in patients with higher degrees of urinary protein leak [475], clarification of this effect by recruiting subjects with >3g/24hrs of proteinuria (with maintained serum albumin concentrations) would be important.

2. Natriuretic effect of ETB receptor

To demonstrate the natriuretic effect of ETB receptor activation, a further study of low dose exogenous ET-1 administration in the presence of high grade ETA blockade is required.

3. ETA-ACEI interaction - bradykinin

Having demonstrated that this interaction is ETB dependent and NO mediated, because ACE inhibitors also block the breakdown of bradykinin, bradykinin receptor antagonism would be useful to delineate whether the interaction with ACE inhibition is acting via a reduction in angiotensin or an increase in bradykinin.

4. ETA-ACEI interaction - renal patients

To clarify if the greater effect of ETA antagonism in renal patients is due to an interaction between ETA receptor blockade and ACE inhibition, or because of an altered activity of the ET systemic in renal disease, a study needs to be performed with ETA receptor antagonists in patients on and off ACE inhibition. Recruitment will need to take into account the requirement for equal control of blood pressure in study phases.

5. Salt sensitivity

Study 7 demonstrated a link between salt sensitivity and the response to ETA receptor antagonism on a high salt diet. However, this was a small scale study, and only one subject exhibited a high degree of salt-sensitivity. Examining the effects of ET receptor antagonism in two hypertensive groups, one clearly identified as salt-sensitive, one not, on both high and low salt diets would clarify the role of ET, particularly of renal origin in salt sensitivity. Because salt sensitivity may be linked to a blunted increase in endothelial NO in response to a high salt diet [426], the comparison of ETA with ETA/B blockade may be interesting in this respect.

6. Chronic ETA receptor blockade in patients with renal impairment

Given the renoprotective profile demonstrated in our studies in renal patients, this needs to be confirmed in chronic studies, with particular attention to any slowing of the rate of progression of renal impairment, or reduction in proteinuria as a surrogate marker [476].

7. Renal effects of ET receptor antagonism in patients with chronic heart failure

Renal dysfunction occurs in CHF partly to reduced renal perfusion pressure. Its presence is a poor prognostic marker [477]. Animal studies have suggested that ET receptor antagonism may improve renal function in CHF. However, despite many studies in man into the role of ET receptor antagonism in heart failure, many with non-selective antagonists, none have specifically examined the effects on renal function. Given that these studies suggest that ETB blockade will abolish any beneficial effects of ETA-ACE interaction both systemically and in respect of renal haemodynamic and tubular function, a clearer picture of the renal effects of ET receptor antagonism should be sought in both acute and chronic studies in this condition characterised by vasoconstriction and sodium retention.

8. Acute intervention in scleroderma hypertensive crisis

Scleroderma associated pulmonary hypertension responds to ET receptor blockade [268, 269]. Though not directly studied in this thesis, given the vasoactive nature of these drugs, and the pro-fibrotic effects of ET-1 experimentally, this condition would represent a potential target for ETA receptor antagonists, particularly given that the current treatment for this condition is ACE inhibition.

9. Studies with cyclosporin

The ability of ET receptor antagonism to influence chronic CyA induced endothelial dysfunction should be explored. However, a model of acute administration to healthy subjects is not applicable for these purposes.

Additionally, though CyA induced hypertension is likely to be multi-factorial, the endothelial dysfunction and sympathetic activation might be amenable to treatment with nebivolol, a beta-adrenoreceptor blocker with nitric oxide donor capabilities.

References

1. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332:411-5.

2. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Nat Acad of Sci* 1989; 86:2863-7.

3. Arinami T, Ishikawa M, Inoue A, Yanagisawa M, Masaki T, Yoshida MC, Hamaguchi H. Chromosomal assignments of the human endothelin family genes: The endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. *Am J Hum Genet* 1991; 48:990-6.

4. Kloog Y, Ambar I, Sokolovsky M, Kochva E, Bdolah A. Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science* 1988; 242:268-70.

5. Howard PG, Plumpton C, Davenport AP. Anatomical localization of pharmacological activity of mature endothelins and their precursors in human vascular tissue. *J Hypertens* 1992; 10:1379-86.

6. Kanse SM, Takahashi K, Warren JB, Perera T, Porta M, Ghatei M, Bloom SR. Production of endothelin by vascular smooth muscle cells. *J Cardiovasc Pharmacol* 1991; 17 Suppl 7:S113.

7. Matsumoto H, Suzuki N, Onda H, Fujino M. Abundance of endothelin-3 in rat intestine, pituitary gland and brain. *Biochem Biophys Res Commun* 1989; 164:74-80.

8. Takahashi K, Jones PM, Kanse SM, Lam HC, Spoke RA, Ghatei MA, Bloom SR. Endothelin in the gastrointestinal tract. Presence of endothelin like immunoreactivity, endothelin-1 messenger RNA, endothelin receptors, and pharmacological effect. *Gastroenterology* 1990; 99:1660-7.

9. Eid H, de Bold ML, Chen JH, de Bold AJ. Epicardial mesothelial cells synthesize and release endothelin. *J Cardiovasc Pharmacol* 1994; 24:715-20.

10. Plumpton C, Champeny R, Ashby M, Kuc RE, Davenport AP. Characterisation of endothelial isoforms in human heart: endothelin-2 demonstrated. *J Cardiovasc Pharmacol* 1993; 22:S26-8.

11. Karet FE, Davenport AP. Localization of endothelin peptides in human kidney. *Kidney Int* 1996; 49:382-7.

12. Kohan DE. Endothelins in the kidney: physiology and pathophysiology. *Am J Kid Dis* 1993; 22:493-510.

13. Kohan DE, Padilla E. Endothelin-1 production by rat inner medullary collecting duct: effect of nitric oxide, cGMP, and immune cytokines. *Am J Physiol* 1994; 266:F291-7.

14. Davenport AP, Hoskins SL, Kuc RE, Plumpton C. Differential distribution of endothelin peptides and receptors in human adrenal gland. *Histochem J* 1996; 28:779-89.

15. Springall DR, Howarth PH, Counihan H, Djukanovic R, Holgate ST, Polak JM. Endothelin immunoreactivity of airway epithelium in asthmatic patients. *Lancet* 1991; 337:697-701.

16. Ehrenreich H, Anderson RW, Fox CH, et al. Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. *J Exp Med* 1990; 172:1741-8.

17. Zhang WW, Badonic T, Hoog A, Jiang MH, Ma KC, Nie XJ, Olsson Y. Astrocytes in Alzheimer's disease express immunoreactivity to the vaso-constrictor endothelin-1. *J Neurol Sci* 1994; 122:90-6.

18. Inagaki H, Bishop AE, Yura J, Polak JM. Localization of endothelin-1 and its binding sites to the nervous system of the human colon. *J Cardiovasc Pharmacol* 1991; 17 Suppl 7:S455-7.

19. Fuxe K, Tinner B, Staines W, Hemsen A, Hersh L, Lundberg JM. Demonstration and nature of endothelin-3-like immunoreactivity in somatostatin and choline acetyltransferase-immunoreactive nerve cells of the neostriatum of the rat. *Neurosci Let* 1991; 123:107-11.

20. Emori T, Hirata Y, Ohta K, et al. Cellular mechanisms of endothelin-1 release by angiotensin and vasopressin. *Hypertension* 1991; 18:165-70.

21. Imai T, Hirata Y, Emori T, Yanagisawa M, Masaki T, Marumo F. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. *Hypertension* 1992; 19:753-7.

22. Ito H, Hirata Y, Adachi S, et al. Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. *J Clin Invest* 1993; 92:398-403.

23. Hahn AW, Resink TJ, Scott-Burden T, Powell J, Dohi Y, Buhler FR. Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. *Cell Regul* 1990; 1:649-59.

24. Zoja C, Orisio S, Perico N, et al. Constitutive expression of endothelin gene in cultured human mesangial cells and its modulation by transforming growth factor-beta, thrombin, and a thromboxane A2 analogue. *Lab Invest* 1991; 64:16-20.

25. Lundberg JM, Ahlborg G, Hemsen A, et al. Evidence for release of endothelin-1 in pigs and humans. *J Cardiovasc Pharmacol* 1991; 17 Suppl 7:S350-3.

26. Vemulapalli S, Chiu P, J., Rivelli M, Foster CJ, Sybertz EJ. Modulation of circulating endothelin levels in hypertension and endotoxemia in rats. *J Cardiovasc Pharmacol* 1991; 18:895-903.

27. Dikranian K, Tomlinson A, Loesch A, Winter R, Burnstock G. Increase in immunoreactivity to endothelin-1 in the mucosal vasculature and epithelium of the large intestine during chronic hypoxia. *J Anat* 1994; 185:609-15.

28. Oliver FJ, de la Rubia G, Feener E, et al. Stimulation of endothelin-1 gene expression by insulin in endothelial cells. *J Biol Chem* 1991; 266:23251-6.

29. Ong AC, Jowett TP, Moorhead JF, Owen JS. Human high density lipoproteins stimulate endothelin-1 release by cultured human renal proximal tubular cells. *Kidney Int* 1994; 46:1315-21.

30. Ong AC, Jowett TP, Scoble JE, O'Shea JA, Varghese Z, Moorhead JF. Effect of cyclosporin A on endothelin synthesis by cultured human renal cortical epithelial cells. *Nephrol Dial Transplant* 1993; 8:748-53.

31. Prins BA, Hu R-M, Nazario B, al. e. Prostaglandin E2 and prostacyclin inhibit the producion and secretion of endothelin from cultured endothelial cells. *J Biol Chem 1994* 1994; 269:11938-44.

32. Boulanger C, Luscher TF. Release of endothelin from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990; 85:587-90.

33. Kohno M, Yokokawa K, Horio T, Yasunari K, Murakawa K, Takeda T. Atrial and brain natriuretic peptides inhibit the endothelin-1 secretory response to angiotensin II in porcine aorta. *Circulation Res* 1992; 70:241-7.

34. Morey AK, Razandi M, Pedram A, Hu RM, Prins BA, Levin ER. Oestrogen and progesterone inhibit the stimulated production of endothlin-1. *Biochem J* 1998; 330:1097-105.

35. Matsumura Y, Hisaki K, Takaoka M, Morimoto S. Phosphoramidon, a metalloproteinase inhibitor, suppresses the hypertensive effect of big endothelin-1. *Eur J Pharmacol* 1990; 185:103-6.

36. Haynes WG, Webb DJ. Endothelin as a regulator of cardiovascular function in health and disease. *J Hypertens* 1998; 16:1081-98.

37. Xu D, Emoto N, Giaid A, Slaughter C, Kaw S, deWit D, Yanagisawa M. ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. *Cell* 1994; 78:473-85.

38. Schweizer A, Valdernaire O, Nelbock P, Deuschle U, Dumas Milne Edwards JB, Stumpf JG, Loffler BM. Human endothelin-converting enzyme (ECE-1): three isofroms with distinct subcelluar localizations. *Biochem J* 1997; 328:871-7.

39. Valdenaire O, Lepailleur-Enouf D, Egidy G, et al. A fourth isoform of endothelin-converting enzyme (ECE-1) is generated from an additional promoter. *Eur J Biochem* 1999; 264:341-9.

40. Emoto N, Yanagisawa M. Endothelin-converting enzyme-2 is a membrane bound, phosphoramidon-sensitive metalloprotease with acidic pH optimum. *J Biol Chem* 1995; 270:15262-8.

41. Ohnaka K, Takayanagi R, Yamauchi T, Okazaki H, Ohashi M, Umeda F, Nawata H. Identification and characterization of endothelin converting activity in cultured bovine endothelial cells. *Biochem Biophys Res Commun* 1990; 168:1128-36.

42. Wypij DM, Nichols JS, Novak PJ, Stacy DL, Berman J, Wiseman JS. Role of mast cell chymase in the extracellular processing of big-endothelin-1 to endothelin-1 in the perfused rat lung. *Biochem Pharmacol* 1992; 43:845-53.

43. Kishi F, Minami K, Okishima N, et al. Novel 31-amino-acid-length endothelins cause constriction of vascular smooth muscle. *Biochem Biophys Res Commun* 1998; 248:387-90.

44. Yoshimoto S, Ishizaki Y, Sasaki T, Murota S. Effect of carbon dioxide and oxygen on endothelin production by cultured porcine cerebral endothelial cells. *Stroke* 1991; 22:378-83.

45. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* 1990; 348:730-2.

46. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, Masaki T. Cloning of a cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. *Nature* 1990; 348:732-5.

47. Hori S, Komatsu Y, Shigemoto R, Mizuno N, Nakanishi S. Distinct tissue distribution and cellular location of two messenger ribonucleic acids encoding different subtypes of rat endothelin receptors. *Endocrinol* 1992; 130:575-582.

48. Davenport AP, O'Reilly G, Molenaar P, et al. Human endothelin receptors characterized using reverse transcriptase-polymerase chain reaction, in situ hybridization, and subtype-selective ligands BQ123 and BQ3020: evidence for expression of ETB receptors in human vascular smooth muscle. *J Cardiovasc Pharmacol* 1993; 22:S22-S25.

49. Ogawa Y, Nakao K, Arai H, et al. Molecular cloning of a non-isopeptide-selective human endothelin receptor. *Biochem Biophys Res Commun* 1991; 178:248-55.

50. Tsukahara H, Ende H, Magazine HI, Bahou WF, Goligorsky MS. Molecular and functional characterization of the non-isopeptide-selective ETB receptor in endothelial cells: receptor coupling to nitric oxide synthase. *J Biol Chem* 1994; 269:21778-85.

51. DeNucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, Vane JR. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 1988; 85:9797-800.

52. Karet F, Kuc R, Davenport A. Novel ligands BQ 123 and BQ 3020 characterize endothelin receptor subtypes ETA and ETB in human kidney. *Kidney Int* 1993; 44:36-42.

53. Thomas CP, Baldi E, Simonson MS, Kester M, Dunn MJ. Endothelin receptors and coupled GTP-binding proteins in glomerular mesangial cells. *J Cardiovasc Pharmacol* 1991; 17 Suppl 7:S79-84.

54. Galron R, Bdolah A, Kloog Y, Sokolovsky M. Endothelin/sarafotoxin receptor induced phosphoinositide turnover: effects of pertussis and cholera toxins and of phorbol ester. *Biochem Biophys Res Commun* 1990; 171:949-54.

55. Baldi E, Musial A, Kester M. Endothelin stimulates phosphatidylcholine hydrolysis through both PLC and PLD pathways in mesangial cells. *Am J Physiol* 1994; 266:F957-65.

56. Griendling KK, Tsuda T, Alexander RW. Endothelin stimulates diacylglycerol accumulation and activates protein kinase C in cultured smooth muscle cells. *J Biol Chem* 1989; 264:8237-40.

57. Mitsuhashi T, Morris RC, Jr., Ives HE. Endothelin-induced increases in vascular smooth muscle Ca++ do not depend on dihydropyridine-sensitive Ca++ channels. *J Clin Invest* 1989; 84:635-9.

58. Simonson MS, Osanai T, Dunn MJ. Endothelin isopeptides evoke Ca2+ signaling and oscillations of cytosolic free [Ca2+] in human mesangial cells. *Biochim Biophys Acta* 1990; 1055:63-8.

59. Cirino M, Martin E, Yano M, Rodger IW. Cardiovascular and renal actions of the endothelin (B) receptor in pigs. *J Cardiovasc Physiol* 1997; 29:704-12.

60. Yanagisawa M, Inoue A, Ishikawa T, et al. Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. *Proc Natl Acad Sci U S A* 1988; 85:6964-7.

61. Noguchi K, Ishikawa K, Yano M, Ahmed A, Cortes A, Abraham WM. Endothelin-1 contributes to antigen-induced airway hyperresponsiveness. *J Appl Physiol* 1995; 79:700-5.

62. Westfall TD, McCafferty GP, Pullen M, et al. Effect of endothelin on bladder contraction: potential role in bladder hyperactivity. *Pharmacology* 2003; 69:7-11.

63. Sorokin A, Kohan DE. Physiology and pathology of endothelin-1 in renal mesangium. *Am J Physiol Renal Physiol* 2003; 285.:F579-89.

64. Kohno M, Horio T, Yokokawa K, Yasunari K, Kurihara N, Takeda T. Endothelin modulates the mitogenic effect of PDGF on glomerular mesangial cells. *Am J Physiol* 1994; 266:F894-900.

65. Tsuboi R, Sato C, Shi CM, Nakamura T, Sakurai T, Ogawa H. Endothelin-1 acts as an autocrine growth factor for normal human keratinocytes. *J Cell Physiol* 1994; 159:213-20.

66. Matsuura A, Yamochi W, Hirata K, Kawashima S, Yokoyama M. Stimulatory interaction between vascular endothelial growth factor and endothelin-1 on each gene expression. *Hypertension* 1998; 32:89-95.

67. Sampaio AL, Rae GA, Henriques MG. Effects of endothelin ETA receptor antagonism on granulocyte and lymphocyte accumulation in LPS-induced inflammation. *J Leukoc Biol* 2004; 76:210-6.

68. Yang LL, Gros R, Kabir MG, Sadi A, Gotlieb AI, Husain M, Stewart DJ. Conditional cardiac overexpression of endothelin-1 induces inflammation and dilated cardiomyopathy in mice. *Circulation* 2004; 109:255-61.

69. Matsushima H, Yamada N, Matsue H, Shimada S. The effects of endothelin-1 on degranulation, cytokine, and growth factor production by skin-derived mast cells. *Eur J Immunol* 2004; 34:1910-9.

70. Didier N, Romero IA, Creminon C, Wijkhuisen A, Grassi J, Mabondzo A. Secretion of interleukin-1beta by astrocytes mediates endothelin-1 and tumour necrosis factor-alpha effects on human brain microvascular endothelial cell permeability. *J Neurochem* 2003; 86:246-54.

71. Hafizi S, Wharton J, Chester A, Yacoub M. Profibrotic Effects of Endothelin-1 via the ET(A) Receptor in Cultured Human Cardiac Fibroblasts. *Cell Physiol Biochem* 2004; 14:285-92.

72. Garcia R, Lachance D, Thibault G. Positive inotropic action, natriuresis and atrial natriuretic factor release induced by endothelin in the conscious rat. *J Hypertens* 1990; 8:725-31.

73. Kawaguchi H, Sawa H, Yasuda H. Endothelin stimulates angiotensin I to angiotensin II conversion in cultured pulmonary artery endothelial cells. *J Mol Cell Cardiol* 1990; 22:839-42.

74. Marriott DB. Endothelin-1 stimulation of noradrenaline and adrenaline release from adrenal chromaffin cells. *Biochem Pharmacol* 1991; 41:521-26.

75. Oishi R, Nonoguchi H, Tomita K, Marumo F. Endothelin-1 inhibits AVP-stimulated osmotic water permeability in rat inner medullary collecting duct. *Am J Physiol* 1991; 261:F951-6.

76. Rossi GP, Ganzaroli C, Cesari M, Maresca A, Plebani M, Nussdorfer GG, Pessina AC. Endothelin receptor blockade lowers plasma aldosterone levels via different mechanisms in primary aldosteronism and high-to-normal renin hypertension. *Cardiovasc Res.* 2003; 57:277-83.

77. Brown MA, Smith PL. Endothelin: a potent stimulator of intestinal ion secretion in vitro. *Regulat Peptides* 1991; 36:1-19.

78. Blouquit S, Sari A, Lombet A, D'herbomez M, Naline E, Matran R, Chinet T. Effects of endothelin-1 on epithelial ion transport in human airways. *Am J Respir Cell Mol Biol.* 2003; 29:245-51.

79. Garvin J, Sanders K. Endothelin inhibits fluid and bicarbonate transport in part by reducing Na+/K+ ATPase activity in the rat proximal straight tubule. *J Am Soc Nephrol* 1991; 2:976-82.

80. Kohan DE. Autocrine role of endothelin in rat inner medullary collecting duct: inhibition of AVP-induced cAMP accumulation. *J Cardiovasc Pharmacol* 1993; 22 Suppl 8:S174-9.

81. Guntupalli J, DuBose TD, Jr. Effects of endothelin on rat renal proximal tubule Na(+)-Pi cotransport and Na+/H+ exchange. *Ame J Physiol* 1994; 266:F658-66.

82. Rossi NF. Regulation of vasopressin secretion by ETA and ETB receptors in compartmentalized rat hypothalamo-neurohypophysial explants. *Am J Physiol Endocrinol Metab* 2004; 286:E535-41.

83. Khodorova A, Navarro B, Jouaville LS, et al. Endothelin-B receptor activation triggers an endogenous analgesic cascade at sites of peripheral injury. *Nat Med* 2003; 9:1055-61.

84. Kurihara Y, Kurihara H, Suzuki H, et al. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. *Nature* 1994; 368:703-10.

85. Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, Yanagisawa M. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 1994; 79:1267-76.

86. Gasic S, Wagner OF, Vierhapper H, Nowotny P, Waldhausl W. Regional haemodynamic effects and clearance of endothelin-1 in humans: renal and peripheral tissues may contribute to overall disposal of the peptide. *J Cardiovasc Pharmacol* 1992; 19:176-80.

87. Dupuis J, Stewart DJ, Cernacek P, Gosselin G. Human pulmonary circulation is an important site for both clearance and production of endothelin-1. *Circulation* 1996; 94:1278-84.

88. Burkhart M, Barton M, Shaw SG. Receptor- and non-receptor-mediated clearance of big-endothelin and endothelin-1: differential effects of acute and chronic ETA blockade. *J Hypertens* 2000; 18:273-9.

89. Abassi ZA, Golomb E, Bridenbaugh R, Keiser HR. Metabolism of endothelin-1 and big endothelin-1 by recombinant neutral endopeptidase EC.3.4.24.11. *Br J Pharmacol* 1993; 109:1024-8.

90. Plumpton C, Haynes WG, Webb DJ, Davenport AP. Phosphoramidon inhibition of the *in vivo* conversion of big endothelin-1 to endothelin-1 in human forearm. *Br J Pharmacol* 1995; 116:1821-8.

91. Plumpton C, Ferro CJ, Haynes WG, Webb DJ, Davenport AP. The increase in human plasma immunoreactive endothelin but not big endothelin-1 or its C-terminal fragment induced by systemic administration of the endothelin antagonist TAK-044. *Br J Pharmacol* 1996; 119:311-4.

92. Benigni A, Perico N, Gaspari F, Zoja C, Bellizzi M, Gabanelli M, Remuzzi G. Increased renal endothelin production in rats with reduced renal mass. *Am J Physiol* 1991; 260:F331-9.

93. Ihara M, Ishikawa K, Fukuroda T, et al. In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ETA receptor. *J Cardiovasc Pharmacol* 1992; 20 Suppl 12:S11-4.

94. Ihara M, Yamanaka R, Ohwaki K, et al. [3H]BQ-123, a highly specific and reversible radioligand for the endothelin ETA receptor subtype. *Eur J Pharmacol* 1995; 274:1-6.

95. Kusumoto K, Fujiwara A, Ikeda S, Watanabe T, Fujino M. Pharmacological characterization of cardiovascular responses induced by endothelin-1 in the perfused rat heart. *Eur J Pharmacol* 1996; 296:65-74.

96. Ishikawa K, Ihara M, Noguchi K, et al. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Nat Acad Sci* 1994; 91:4892-6.

97. Fukami T, Niiyama K, Amano Y, Hisaka A, Fujino N. Cyclic pentapeptide endothelin A receptor antagonists with attenuated in vivo clearance. *Chem Pharmaceut Bull* 1996; 44:609-14.

98. Clozel M, Breu V, Gray GA, et al. Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J Pharmacol Exp Ther* 1994; 270:228-35.

99. Ohlstein EH, Nambi P, Douglas SA, et al. SB 209670, a rationally designed potent nonpeptide endothelin receptor antagonist. *Proc Natl Acad Sci U S A* 1994; 91:8052-6.

100. Ohlstein EH, Nambi P, Lago A, et al. Nonpeptide endothelin receptor antagonists. VI: Pharmacological characterization of SB 217242, a potent and highly bioavailable endothelin receptor antagonist. *J Pharmacol Exp Ther.* 1996; 276:609-15.

101. Liu G, Henry KJJ, Szczepankiewicz BG, et al. Pyrrolidine-3-carboxylic acids as endothelin antagonists. 3. Discovery of a potent, 2-nonaryl, highly selective ETA antagonists (A-216546). *J Med Chem* 1998; 41:3261-75.

102. Wu C, Chan MF, Stavros F, et al. Discovery of TBC-11251, a potent, long-acting, orally active endothelin receptor-A selective antagonist. *J Med Chem* 1997; 40:1690-7.

103. Hoshino T, Yamauchi R, Kikkawa K, Yabana H, Murata S. Pharmacological profile of T-0201, a highly potent and orally active endothelin receptor antagonist. *J Pharmacol Exp Therapeut* 1998; 286:643-9.

104. Webb ML, Meek TD. Inhibitors of endothelin. *Med Res Rev* 1997; 17:17-67.

105. Kohan DE. Endothelin synthesis by rabbit tubule cells. *Am J Physiol* 1991; 261:F221-6.

106. Kasinath BB, Fried TA, Davalath S, Marsden PA. Glomerular epithelial cells synthesize endothelin peptides. *Am J Pathol* 1992; 141:279-83.

107. Kohan DE. Production of endothlein-1 by rat mesangial cells. *J Lab Clin Med* 1992; 119:477-84.

108. Kohan DE, Padilla E. Osmolar regulation of endothelin-1 production by rat inner medullary collecting duct. *J Clin Invest* 1993; 91:1235-40.

109. Clavell AL, Burnett JCJ. Physiologic and pathophysiologic roles of endothelin in the kidney. *Curr Opin Nephrol Hypertens* 1994; 3:66-72.

110. Badr KF, Murray JJ, Breyer MD, Takahashi K, Inagami T, Harris RC. Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. Elucidation of signal transduction pathways. *J Clin Invest 1989* 1989; 86:336-42.

111. Miura K, Yukimura T, Yamashita Y, Shimmen Y, Okumura M, Imanishi M, Yamamoto K. Endothelin stimulates the renal production of prostaglandin E2 and I2 in anaesthetized dogs. *Eur J Pharmacol* 1989; 170:91-3.

112. Katoh T, Chang H, Uchida S, Okuda T, Kurokawa K. Direct effects of endothelin in the rat kidney. *Am J Physiol* 1990; 258:F397-402.

113. Chou SY, Porush JG. Renal actions of endothelin-1 and endothelin-3: Interactions with the prostaglandin system and nitric oxide. *Ame J Kid Dis* 1995; 26:116-23.

114. Pernow J, Franco-Cereceda A, Matran R, Lundberg JM. Effect of endothelin-1 on regional vascular resistance in the pig. *J Cardiovasc Pharmacol* 1989; 13 Suppl 16:S205-6.

115. Rubinstein I, Gurbanov K, Hoffman A, Better OS, Winaver J. Differential effect of endothelin-1 on renal regional blood flow: role of nitric oxide. *J Cardiovasc Pharmacol* 1995; 26:S208-10.

116. Denton KM, Shweta A, Finkelstein L, Flower RL, Evans RG. Effect of endothelin-1 on regional kidney blood flow and renal arteriole calibre in rabbits. *Clin Exp Pharmacol Physiol* 2004; 31:494-501.

117. Gurbanov K, Rubinstein I, Hoffman A, Abassi Z, Better OS, Winaver J. Differential regulation of renal regional blood flow by endothelin-1. *Am J Physiol* 1996; 271:F1166-72.

118. Rabelink TJ, Kaasjager KAH, Boer P, Stroes EG, Braam B, Koomans HA. Effects of endothelin-1 on renal function in humans. Implications for physiology and pathophysiology. *Kidney Int* 1994; 46:376-81.

119. Sorensen SS, Madsen JK, Pedersen EB. Systemic and renal effects of intravenous infusion of endothelin-1 in healthy human volunteers. *Am J Physiol* 1994; 266:F411-8.

120. Vuurmans JL, Boer P, Koomans HA. Effects of endothelin-1 and endothelin-1-receptor blockade on renal function in humans. *Nephrol Dial Transplant* 2004; Aug 24 [Epub ahead of print].

121. Clavell AL, Stingo AJ, Marguiles KB, Brandt RR, Burnett JCJ. Role of endothelin receptor subtypes in the in vivo regulation of renal function. *Am J Physiol* 1995; 268:F455-60.

122. Ignasiak DP, McClanahan TB, Saganek LJ, Potoczak RE, Hallak H, Gallagher KP. Effects of endothelin ETA receptor antagonism with PD156707 on haemodynamics and renal vascular resistance in rabbits. *Eur J Pharmacol* 1997; 321:295-300.

123. Brooks DP, DePalma PD, Pullen M, Elliot JD, Ohlstein EH, Nambi P. SB 234551, a novel endothelin-A receptor antagonist, unmasks endothelin-induced renal vasodilatation in the dog. *J Cardiovasc Pharmacol* 1998; 31 Suppl 1:S339-41.

124. Yukimura T, Yamashita Y, Miura K, Kim S, Iwao.H., Takai H, Okada T. Renal vasodilating actions of selective endothelin ETB receptor agonist, IRL 1620. *Eur J Pharmacol* 1994; 264:399-405.

125. Bigaud M, Pelton JT. Discrimination between ETA- and ETB-receptor-mediated effects of endothelin-1 by BQ-123 in the anaesthetized rat. *Br J Pharmacol* 1992; 107:912-8.

126. Wellings RP, Warner TD, Thiemermann C, Cristol JP, Corder R, Vane JR. Vasoconstriction in the rat kidney induced by endothelin-1 is blocked by PD 145065. *J Cardiovasc Pharmacol* 1993; 22 Suppl 8:S103-6.

127. Wellings RP, Corder R, Warner TD, Cristol JP, Thiemermann C, Vane JR. Evidence from receptor antagonists of an important role for ETB receptor-mediated vasoconstrictor effects of endothelin-1 in the rat kidney. *Br J Pharmacol* 1994; 111:515-20.

128. Kitamura K, Tanaka T, Kato J, Eto T, Tanaka K. Regional distribution of immunoreactive endothelin in porcine tissue: abundance in inner medulla of kidney. *Biochem Biophys Res Commun* 1989; 161:348-52.

129. Morita S, Kitamura K, Yamamoto Y, et al. Immunoreactive endothelin in human kidney. *Ann Clin Biochem* 1991; 28:267-71.

130. Plato CF, Pollock DM, Garvin JL. Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release. *Am J Physiol Renal Physiol* 2000; 279:F326-33.

131. Herrera M, Garvin JL. Endothelin stimulates endothelial nitric oxide synthase expression in the thick ascending limb. *Am J Physiol Renal Physiol* 2004; 287:F231-5.

132. Gallego MS, Ling BN. Regulation of amiloride-sensitive Na+ channels by endothelin-1 in distal nephron cells. *Am J Physiol* 1996; 271:F451-60.

133. Vassileva I, Mountain C, Pollock DM. Functional role of ETB receptors in the renal medulla. *Hypertension* 2003; 41:1359-63.

134. Webb DJ, Monge JC, Rabelink TJ, Yanagisawa M. Endothelin: new discoveries and rapid progress in the clinic. *Trend Pharmacol Sci* 1998; 19:5-8.

135. Kotelevtsev K, Webb DJ. Endothelin as a natriuretic hormone: the case for a paracrine action mediated by nitric oxide. *Cardiovasc Res* 2001; 51:481-8.

136. Ahn D, Ge Y, Stricklett PK, et al. Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. *J Clin Invest. 2004 Aug;114(4):504-11.* 2004; 114:504-11.

137. Kaasjager KAH, Shaw S, Koomans HA, Rabelink TJ. Role of endothelin receptor subtypes in the systemic and renal response to endothelin-1 in humans. *J Am Soc Nephrol* 1997; 8:32-9.

138. Weitzberg E, Hemsen A, Lundberg JM, Ahlborg G. ET-3 is extracted by and induces potent vasoconstriction in human splanchnic and renal vasculatures. *J Appl Physiol* 1995; 79:1255-9.

139. Schmetter L, Dallinger S, Bobr B, Selenko N, Eichler H-G, Woltz M. Systemic and renal effects of an ETA receptor subtype-specific antagonist in healthy subjects. *Br J Pharmacol* 1998; 124:930-4.

140. Honing ML, Hijmering ML, Ballard DE, Yang YP, Padley RJ, Morrison PJ, Rabelink TJ. Selective ET(A) receptor antagonism with ABT-627 attenuates all renal effects of endothelin in humans. *J Am Soc Nephrol* 2000; 11:1498-504.

141. Bohm F, Pernow J, Lindstrom J, Ahlborg G. ETA receptors mediate vasoconstriction, whereas ETB receptors clear endothelin-1 in the splanchnic and renal circulation of healthy men. *Clin Sci* 2003; 104:143-51.

142. Freed MI, Wilson DE, Thompson KA, Harris RZ, Ilson BE, Jorkasky DK. Pharmacokinetics and pharmacodynamics of SB 209670, an endothelin receptor antagonist: Effects on the regulation of renal vascular tone. *Clin Pharmacol Therapeut* 1999; 65:473-82.

143. Shibouta Y, Suzuki N, Shino A, Matsumoto H, Terashita Z-I, Kondo K, Nishikawa K. Pathophysiological role of endothelin in acute renal failure. *Life Sci* 1990; 46:1611-8.

144. Fogo A, Hellings SE, Inagami T, Kon V. Endothelin receptor antagonism is protective in in vivo acute cyclosporine toxicity. *Kidney Int* 1992; 42:770-4.

145. Conger JD, Kim GE, Robinette JB. Effects of ANG II, ETA, and TxA2 receptor antagonists on cyclosporin A renal vasoconstriction. *Am J Physiol* 1994; 267:F 443-9.

146. Abassi ZA, Pieruzzi F, Nakhoul F, Keiser HR. Effects of cyclosporin A on the synthesis, excretion and metabolism of endothelin in the rat. *Hypertension* 1996; 27:1140-8.

147. Krause SM, T.F. W, Greenlee WJ, Ranaen R, Williams DLJ, Kivlighn SD. Renal protection by dual ETA/ETB endothelin antagonists L-754,142, after aortic cross-clamping in the dog. *J Am Soc Nephrol* 1997; 8:1061-71.

148. Lariviere R, D'Amours M, Lebel M, Kingma I, Grose JH, Caron L. Increased immunoreactive endothelin-1 levels in blood vessels and glomeruli of rats with reduced renal mass. *Kidney Blood Press Res* 1997; 20:372-80.

149. Heyman SN, Clark BA, Kaiser N, Spokes K, Rosen S, Brezis M, Epstein FH. Radiocontrast agents induce endothelin release in vivo and in vitro. *J Am Soc Nephrol* 1992; 3:58-65.

150. Margulies KB, Hildebrand FL, Heublein DM, Burnett JCJ. Radiocontrast increases plasma and urinary endothelin. *J Am Soc Nephrol* 1991; 2:1041-5.

151. Fujisaki K, Kubo M, Masuda K, et al. Infusion of radiocontrast agents induces exaggerated release of urinary endothelin in patients with impaired renal function. *Clin Exp Nephrol* 2003; 7:279-83.

152. Brooks DP, DePalma PD. Blockade of radiocontrast-induced nephrotoxicity by the endothelin receptor antagonist, SB 209670. *Nephron* 1996; 72:629-36.

153. Pollock DM, Polakowski JS, Wegner CD, Opgenorth TJ. Beneficial effect of ETA receptor blockade in a rat model of radiocontrast-induced nephropathy. *Ren Fail* 1997; 19:753-61.

154. Liss P CP, Nygren A, Palm F, Hansell P. Et-A receptor antagonist BQ123 prevents radiocontrast media-induced renal medullary hypoxia. *Acta Radiol* 2003; 44:111-7.

155. Wang A, Holcslaw T, Bashore TM, et al. Exacerbation of radiocontrast nephrotoxicity by endothelin receptor antagonism. *Kidney Int* 2000; 57:1675-80.

156. Miller RL, Kohan DE. Hypoxia regulates endothelin-1 production by the inner medullary collecting duct. *J Lab Clin Med 1998 Jan;* 1998; 131:45-8.

157. Wilhelm SM, Stowe NT, Robinson AV, Schulak JA. The use of the endothelin receptor antagonist, tezosentan, before or after renal ischemia protects renal function. *Transplantation* 2001; 71:211-6.

158. Forbes JM, Jandeleit-Dahm K, Allen TJ, Hewitson TD, Becker GJ, Jones CL. Endothelin and endothelin A/B receptors are increased after ischaemic acute renal failure. *Exp Nephrol* 2001; 9:309-16.

159. Birck R, Knoll T, Braun C, Kirchengast M, Munter K, van der Woude FJ, Rohmeiss P. Improvement of postischemic acute renal failure with the novel orally active endothelin-A receptor antagonist LU 135252 in the rat. *J Cardiovasc Pharmacol* 1998; 32:80-6.

160. Knoll T, Schult S, Birck R, et al. Therapeutic administration of an endothelin-A receptor antagonist after acute ischemic renal failure dose-dependently improves recovery of renal function. *J Cardiovasc Pharmacol* 2001; 37:483-8.

161. Huang C, Huang C, Hestin D, Dent PC, Barclay P, Collis M, Johns EJ. The effect of endothelin antagonists on renal ischaemia-reperfusion injury and the development of acute renal failure in the rat. *Nephrol Dial Transplant* 2002; 17:1578-85.

162. Jerkic M, Miloradovic Z, Jovovic D, et al. Relative roles of endothelin-1 and angiotensin II in experimental post-ischaemic acute renal failure. *Nephrol Dial Transplant* 2004; 19:83-94.

163. Inman SR, Burns TE, Plott WK, Pomilee RA, Antonelli JA, Lewis RM. Endothelin receptor blockade during hypothermic perfusion preservation mitigates the adverse effect of preretrieval warm ischemic injury on posttransplant glomerular filtration rate. *Transplantation* 2002; 74:164-8.

164. Herrero I, Torras J, Riera M, et al. Prevention of cold ischaemia-reperfusion injury by an endothelin receptor antagonist in experimental renal transplantation. *Nephrol Dial Transplant* 1999; 14:872-80.

165. Kuro T, Kohnou K, Kobayashi Y, Takaoka M, Opgenorth TJ, Wessale JL, Matsumura Y. Selective antagonism of the ETA receptor, but not the ETB receptor, is protective against ischemic acute renal failure in rats. *Jpn J Pharmacol* 2000; 82:307-16.

166. Nishida M, Ieshima M, Konishi F, Yamashita J, Takaoka M, Matsumura Y. Role of endothelin B receptor in the pathogenesis of ischemic acute renal failure. *J Cardiovasc Pharmacol* 2002; 40:586-93.

167. Forbes JM, Hewitson TD, Becker GJ, Jones CL. Simultaneous blockade of endothelin A and B receptors in ischemic acute renal failure is detrimental to long-term kidney function. *Kidney Int* 2001; 59:1333-41.

168. Taler SJ, Textor SC, Canzanello VJ, Schwartz L. Cyclosporin-induced hypertension: incidence, pathogenesis and management. *Drug Saf* 1999; 20:437-49.

169. Bunchman TE, Brookshire CA. Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. *J Clin Invest* 1991; 88:310-4.

170. Haug C, Duell T, Voisard R, et al. Cyclosporine A stimulates endothelin release. *J Cardiovasc Pharmacol* 1995; 26 Suppl 3:S239-41.

171. Hutchinson IV. An endothelin-transforming growth factor beta pathway in the nephrotoxicity of immunosuppressive drugs. *Curr Opin Nephrol Hypertens* 1998; 7:665-71.

172. Rezzani R, Rodella L, Bianchi R. Induction of endothelin in rat kidney after cyclosporine A treatment. *Acta Histochem* 2001; 103:423-31.

173. Karet FE, Davenport AP. Selective downregulation of ETA receptor mRNA in renal transplant recipients on cyclosporin A revealed by quantitive RT-PCR. *Nephrol Dial Transplant* 1996; 11:1976-82.

174. Brooks DP, Contino LC. Prevention of cyclosporine A-induced renal vasoconstriction by the endothelin receptor antagonist SB 209670. *Eur J Pharmacol* 1995; 294:571-6.

175. Kon V, Hunley TE, Fogo A. Combined antagonism of endothelin A/B receptors links endothelin to vasoconstriction whereas angiotensin II effects fibrosis. Studies in chronic cyclosporine nephrotoxicity in rats. *Transplantation* 1995; 60:89-95.

176. Prevot A, Semama DS, Tendron A, Justrabo E, Guignard JP, Gouyon JB. Endothelin, angiotensin II and adenosine in acute cyclosporine A nephrotoxicity. *Pediatr Nephrol* 2000; 14:927-34.

177. Binet I, Wallnoffer A, Weber C, Jones R, Thiel G. Renal haemodynamics and pharmacokinetics of bosentan with and without cyclosporine A. *Kidney Int* 2000:224-31.

178. Slowinski T, Subkowski T, Diehr P, Bachert D, Fritsche L, Neumayer HH, Hocher B. Interaction of the endothelin system and calcineurin inhibitors after kidney transplantation. *Clin Sci* 2002; 103 Suppl 48:396-8S.

179. Kocik M, Malek I, Glagolicova A, Pirk J. The effect of cyclosporin A on the level of big endothelin in patients one year after orthotopic heart transplantation. *Transpl Int* 2004; 17:65-70.

180. Brooks DP, Contino LC, Storer B, Ohlstein EH. Increased endothelin excretion in rats with renal failure induced by partial nephrectomy. *Br J Pharmacol* 1991; 140:987-9.

181. Orisio S, Benigni A, Bruzzi I, et al. Renal endothelin gene expression is increased in remnant kidney and correlates with disease progression. *Kidney Int* 1993; 43:354-8.

182. Brochu E, Lacasse S, Moreau C, Lebel M, Kingma I, Grose JH, Lariviere R. Endothelin ET(A) receptor blockade prevents the progression of renal failure and hypertension in uraemic rats. *Nephrol Dial Transplant* 1999; 14:1881-8.

183. Nabokov A, Amann K, Wagner J, Gehlen F, Munter K, Ritz E. Influence of specific and non-specific endothelin receptor antagonists on renal morphology in rats with surgical renal ablation. *Nephrol Dial Transplant* 1996; 11:514-20.

184. Shimizu T, Hata S, Kuroda T, Mihara S, Fujimoto M. Different roles of two types of endothelin receptors in partial ablation-induced chronic renal failure in rats. *Eur J Pharmacol* 1999; 381:39-49.

185. Wolf SC, Brehm BR, Gaschler F, et al. Protective effects of endothelin antagonists in chronic renal failure. *Nephrol Dial Transplant* 1999; 14 Suppl 4:29-30.

186. Ong AC, Newby LJ, Dashwood MR. Expression and cellular localisation of renal endothelin-1 and endothelin receptor subtypes in autosomal-dominant polycystic kidney disease. *Nephron Exp Nephrol* 2003; 93:e80.

187. Chen HC, Guh JY, Chang JM, Tsai JC, Hwang SJ, Lai YH. Plasma and urinary endothelin-1 in focal segmental glomerulosclerosis. *J Clin Lab Anal* 2001; 15:59-63.

188. Vlachojannis J, Tsakas S, Petropoulou C, Kurz P. Increased renal excretion of endothelin-1 in nephrotic patients. *Nephrol Dial Transplant* 1997; 12:470-3.

189. Cantaro S, Milan Manani S, Marcon R, Bonfante L, Masiero M, D'Angelo A, Calo L. Urinary excretion of vasoactive substances in chronic renal failure. *Clin Nephrol 2001 May;* 2001; 55:393-9.

190. Honing MLH, Bouter PK, Ballard DE, Padley P, Morrison PJ, Rabelink TJ. ABT-627, a selective ETA-receptor antagonist, reduces proteinuria in patients with diabetes mellitus. Regulation of vascular tone in humans by endothelium-derived mediators. Thesis. Utrecht, 2000:89-102.

191. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet* 1994; 344:852-4.

192. Berrazueta JR, Bhagat K, Vallance P, MacAllister RJ. Dose- and time-dependency of the dilator effects of the endothelin antagonist BQ-123, in the human forearm. *Br J Clin Pharmacol* 1997; 44:569-71.

193. Haynes WG, Hand MF, Dockrell MEC, et al. Physiological role of nitric oxide in regulation of renal function in humans. *Am J Physiol* 1997; 272:F364-71.

194. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation* 1998; 97:752-6.

195. Hand MF, Haynes WG, Webb DJ. Reduced endogenous endothelin-1-mediated vascular tone in chronic renal failure. *Kidney Int* 1999; 55:613-20.

196. Cardillo C, Kilcoyne CM, Waclawiw M, Cannon ROr, Panza JA. Role of endothelin in the increased vascular tone of patients with essential hypertension. *Hypertension* 1999; 33:753-8.

197. Cardillo C, Kilcoyne CM, Cannon ROr, Panza JA. Increased activity of endogenous endothelin in patients with hypercholesterolemia. *J Am Coll Cardiol* 2000; 36:1483-8.

198. Goddard J, Webb DJ. Endothelin antagonists and hypertension: a question of dose? *Hypertension* 2002; 40:e1-2.

199. Montanari A, Biggi A, Carra N, et al. Endothelin-A blockade attenuates systemic and renal hemodynamic effects of L-NAME in humans. *Hypertension* 2000; 35:518-23.

200. Verhaar MC, Grahn AY, van Weerdt AWN, et al. Pharmacokinetics and pharmacodynamic effects of ABT-627, an oral ETA selective endothelin antagonist, in humans. *Br J Clin Pharmacol* 2000; 49:562-73.

201. Haynes WG, Ferro CJ, O'Kane KP, Somerville D, Lomax CC, Webb DJ. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in man. *Circulation* 1996; 93:1860-70.

202. Weber C, Schmitt R, Birnboeck H, et al. Pharmacokinetics and pharmacodynamics of the endothelin-receptor antagonist bosentan in healthy human subjects. *Clin Pharmacol Therapeut* 1996; 60:124-37.

203. Clozel M, Gray GA, Breu W, Loffler B, Osterwalder R. The endothelin ETB receptor mediates both vasodilatation and vasoconstriction in vivo. *Biochem Biophys Res Commun* 1992; 186:867-873.

204. Moreland S, McMullen DM, Delaney CL, Lee VG, Hunt JT. Venous smooth muscle contains vasoconstrictor ETB- like receptors. *Biochem Biophys Res Commum* 1992; 184:100-6.

205. Seo B, Oemar BS, Siebenmann R, von Segesser L, Luscher TF. Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation* 1994; 89:1203-8.

206. Davenport AP, Maguire JJ. Is endothelin-induced vasoconstriction mediated only by ETA receptors in humans? *Trend Pharmacol Sci* 1994; 15:9-11.

207. Haynes WG, Strachan FE, Webb DJ. Endothelin ETA and ETB receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. *Circulation* 1995; 92:357-63.

208. Haynes WG, Strachan FE, Gray GA, Webb DJ. Forearm vasoconstriction to endothelin-1 is mediated by ETA and ETB receptors in vivo in humans. *J Cardiovasc Pharmacol* 1995; 26 Suppl 3:S40-3.

209. Haynes WG, Ferro CE, Webb DJ. Physiologic role of endothelin in maintenance of vascular tone in humans. *J Cardiovasc Pharmacol* 1995; 26 Suppl 3:S183-5.

210. Strachan FE, Spratt JC, Wilkinson IB, Johnston N, Gray GA, Webb DJ. Systemic blockade of the endothelin-B receptor increases peripheral resistance in healthy men. *Hypertension* 1999; 33:581-5.

211. Moravec CS, Reynolds EE, Stewart RW, Bond M. Endothelin is a positive inotropic agent in human and rat heart un vitro. *Biochem Biophys Res Commun* 1989; 159:14-8.

212. Pieske B, Beyermann B, Breu V, et al. Functional effects of endothelin regulation of endothelin receptors in isolated human nonfailing and failing myocardium. *Circulation* 1999; 99:1082-9.

213. Beyer ME, Slesak G, Hovelborn T, Kazmaier S, Nerz S, Hoffmeister M. Inotropic effects of endothelin-1. Interaction with molsidomine and with BQ 610. *Hypertension* 1999; 33:145-52.

214. Schiffrin EL. Endothelin and endothelin antagonists in hypertension. *J Hypertens* 1998; 16:1891-5.

215. Hoffman A, Grossman E, Goldstein DS, Gill JRJ, Keiser H. Urinary excretion rate of endothelin-1 in patients with essential hypertension and salt-sensitivity. *Kidney Int* 1994; 45:556-60.

216. Zoccali C, Leonardis D, Parlongo S, Mallamaci F, Postorino M. Urinary and plasma endothelin-1 in essential hypertension and in hypertension secondary to renoparenchymal disease. *Nephrol Dial Transplant* 1995; 10:1320-3.

217. Hughes AK, Cline RC, Kohan DE. Alterations in renal endothelin-1 production in the spontaneously hypertensive rat. *Hypertension* 1992; 20:666-73.

218. Goddard J, Webb DJ. Plasma endothelin concentrations in hypertension. *J Cardiovasc Pharmacol* 2000; 35 Suppl 1:S25-31.

219. Rossi GP, Colona S, Pavan E, et al. Endothelin-1 and its mRNA in the wall layers of human arteries ex vivo. *Circulation* 1999; 99:1147-55.

220. Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon ROr, Panza JA. Selective defect in nitric oxide synthesis may explain the impaired endothelium-dependent vasodilation in patients with essential hypertension. *Circulation* 1998; 97:851-6.

221. Cardillo C, Kilcoyne CM, Cannon ROr, Panza JA. Attenuation of cyclic nucleotide-mediated smooth muscle relaxation in blacks as a cause of racial differences in vasodilator function. *Circulation* 1999; 99:90-5.

222. Martin P, Ninio D, Krum H. Effect of endothelin blockade on basal and stimulated forearm blood flow in patients with essential hypertension. *Hypertension* 2002; 39:821-4.

223. Campia U, Cardillo C, Panza JA. Ethnic differences in the vasoconstrictor activity of endogenous endothelin-1 in hypertensive patients. *Circulation 2004* 2004; 109:3191-5.

224. Krum H, Viskoper RJ, Lacourciere Y, Budde M, Charlon V. The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. *New Engl J Med* 1998; 338:784-90.

225. Ergul S, Parish DC, Puett P, Ergul A. Racial Differences in Plasma Endothelin-1 Concentrations in Individuals With Essential Hypertension. *Hypertension* 1996; 28:652-5.

226. Koyama H, Tabata T, Nishzawa Y, Inoue T, Morii H, Yamaji T. Plasma endothelin levels in patients with uraemia. *Lancet* 1989; 333:991-2.

227. Warrens AN, Cassidy MJ, Takahashi K, Ghatei MA, Bloom SR. Endothelin in renal failure. *Nephrol Dial Transplant* 1990; 5:418-22.

228. Ohta K, Hirata Y, Shichiri M, Kanno K, Emori T, Tomita K, Marumo F. Urinary excretion of endothelin-1 in normal subjects and patients with renal disease. *Kidney Int* 1991; 39:307-11.

229. Kohno M, Murakawa K, Yasunari K, Yokokawa K, Horio T, Kurihara N, Takeda T. Prolonged blood pressure elevation after endothelin administration in bilaterally nephrectomized rats. *Metab Clin Exp* 1989; 38:712-3.

230. Kanai H, Okuda S, Kiyama S, Tomooka S, Hirakata H, Fujishima M. Effects of endothelin and angiotensin II on renal haemodynamics in experimental mesangial proliferative nephritis. *Nephron* 1993; 64:609-14.

231. Okada Y, Nakata M, Izumoto H, et al. Role of endothelin ETB receptor in partial ablation-induced chronic renal failure in rats. *Eur J Pharmacol* 2004; 494:63-71.

232. Takahashi K, Totsune K, Imai Y, et al. Plasma concentrations of immunoreactive-endothelin in patients with chronic renal failure treated with recombinant human erythropoietin. *Clin Sci* 1993; 84:47-50.

233. Hand MF, Haynes WG, Johnstone HA, Anderton JL, Webb DJ. Erythropoietin enhances vascular responsiveness to norepinephrine in renal failure. *Kidney International* 1995; 48:806-13.

234. Nagai T, Akizawa T, Nakashima Y, et al. Effects of rHuEpo on cellular proliferation and endothelin-1 production in cultured endothelial cells. *Nephrol Dial Transplant* 1995; 10:1814-9.

235. Quaschning T, Ruschitzka F, Stallmach T, et al. Erythropoietin-induced excessive erythrocytosis activates the tissue endothelin system in mice. *FASEB J* 2003; 17:259-61.

236. Hon G, Vaziri ND, Kaupke CJ, Tehranzadeh A, Barton C. Lack of a fast-acting effect of erythropoietin on arterial blood pressure and endothelin level. *Artificial Org* 1995; 19:188-91.

237. Lai KN, Lui SF, Leung JC, Law E, Nicholls MG. Effect of subcutaneous and intraperitoneal administration of recombinant human erythropoietin on blood pressure and vasoactive hormones in patients on continuous ambulatory peritoneal dialysis. *Nephron* 1991; 57:394-400.

238. Carlini R, Obialo CI, Rothstein M. Intravenous erythropoietin (rHuEPO) administration increases plasma endothelin and blood pressure in hemodialysis patients. *Am J Hypertens* 1993; 6:103-7.

239. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC, Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *New Engl J Med* 1991; 325:997-1001.

240. Ihling C, Gobel HR, Lippoldt A, Wessels S, Paul M, Schaefer HE, Zeiher AM. Endothelin-1-like immunoreactivity in human atherosclerotic coronary tissue: a detailed analysis of the cellular distribution of endothelin-1. *J Pathol* 1996; 179:303-8.

241. Hasdai D, Holmes DRJ, Garratt KN, Edwards WD, Lerman A. Mechanical pressure and stretch release endothelin-1 from human atherosclerotic coronary arteries in vivo. *Circulation* 1997; 95:357-62.

242. Kurata C, Callahan RJ, Molea N, Wilkinson R, Fischman AJ, Strauss HW. Localisation of [125I] endothelin-1 in injured aorta of rabbits. *Eur J Pharmacol* 1995; 293:109-14.

243. Fukuda S, Taga K, Tanaka T, Sakuma K, Fujiwara N, Shimoji K, Fujihara H. Relationship between tissue ischaemia and venous endothelin-1 during abdominal aortic aneurysm surgery. *J Cardiothor Vasc Anesthes* 1995; 9:510-4.

244. Schricker K, Scholz H, Hamann M, Clozel M, Kramer BK, Kurtz A. Role of endogenous endothelins in the renin system of normal and two-kidney, one clip rats. *Hypertension* 1995; 25:1025-9.

245. Schreij G, van Es PN, Schiffers PM, De Leeuw PM. Renal arterial and venous endothelin in hypertensive patients with or without renal artery stenosis. *Blood Press* 1994; 3:370-4.

246. Takeda M, Komeyama T, Katayama Y, et al. Dissociation of selective renal venous plasma endothelin-1 activity and corresponding plasma renin activity in a patient with hypertension, severe stenosis of unilateral renal artery and ipsilateral decreased renal function. *Nephron* 1994; 68:372-4.

247. Poch E, Jimenez W, Feu F, et al. Increased plasma endothelin concentration in atherosclerotic renovascular hypertension. *Nephron* 1995; 71:291-6.

248. Januszewicz A, Symonides B, Lapinski M, et al. Endothelin -1 and neuropeptide Y plasma concentrations in renal venous blood of hypertensive patients with unilateral renal artery stenosis. *J Hum Hypertens* 1995; 9:815-20.

249. Teunissen KE, Postma CT, van Jaarsveld BC, Derkx FH, □Thien T. Endothelin and active renin levels in essential hypertension and hypertension with renal artery stenosis before and after percutaneous transluminal renal angioplasty. *J Hypertens* 1997; 12:1791-6.

250. Benigni A, Orisio S, Gaspari F, Frusca T, Amuso G, Remuzzi G. Evidence against a pathogenetic role for endothelin in pre-eclampsia. *Br J Obst Gynaecol* 1992; 99:789-802.

251. Lumme R, Laatikainen T, Vuolteenaho O, Leppaluoto J. Plasma endothelin, atrial natriuretic peptide (ANP) and uterine and umbilical artery flow velocity waveforms in hypertensive pregnancies. *Br J Obst Gynaecol* 1992; 99:761-4.

252. Wolff K, Carlstrom K, Fyhrquist F, Hemsen A, Lunell NO, Nisell H. Plasma endothelin in normal and diabetic pregnancy. *Diabet Care* 1997; 20:653-6.

253. Taylor RN, Varma M, Teng NN, Roberts JM. Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies. *J Clin Endocrinol Metab* 1990; 71:1675-7.

254. Florijn KW, Derkx FH, Visser W, Hofman JA, Rosmalen FM, Wallenburg HC, Schalekamp MA. Plasma immunoreactive endothelin-1 in pregnant women with and without pre-eclampsia. *J Cardiovasc Pharmacol* 1991; 17 Suppl 7:S446-8.

255. Mastrogiannis DS, O'Brien WF, Krammer J, Benoit R. Potential role of endothelin-1 in normal and hypertensive pregnancies. *Am J Obst Gynecol* 1991; 165:1711-6.

256. Nova A, Sibai BM, Barton JR, Mercer BM, Mitchell MD. Maternal plasma level of endothelin is increased in preeclampsia. *Am J Obst Gynecol* 1991; 165:724-7.

257. Schiff E, Ben-Baruch G, Peleg E, Rosenthal T, Alcalcy M, Devir M, Mashiach S. Immunoreactive circulating endothelin-1 in normal and hypertensive pregnancies. *Am J Obst Gynecol* 1992; 166:624-8.

258. Sudo N, Kamoi K, Ishibashi M, Yamaji T. Plasma endothelin-1 and big endothelin-1 levels in women with pre-eclampsia. *Acta Endocrinol* 1993; 129:114-20.

259. Barden A, Beilin LJ, Ritchie J, Walters BN, Michael CA. Plasma and urinary endothelin-1, prostacyclin metabolites and platelet consumption in pre-eclampsia and essential hypertensive pregnancy. *Blood Press* 1994; 3:38-46.

260. Wolff K, Nisell H, Carlstrom K, Kublickiene K, Hemsen A, Lunell NO, Lindblom B. Endothelin-1 and big endothelin 1 levels in normal term pregnancy and preeclampsia. *Regulat Pept* 1996; 67:211-6.

261. Clark BA, Halvorson L, Sachs B, Epstein FH. Plasma endothelin levels in preeclampsia: elevation and correlation with uric acid levels and renal impairment. *Am J Obst Gynecol* 1992; 166:962-8.

262. McMahon LP, Redman CW, Firth JD. Expression of the three endothelin genes and plasma levels of endothelin in pre-eclamptic and normal gestations. *Clin Sci* 1993; 85:417-24.

263. Furuhashi N, Kimura H, Nagae H, Yajima A. Maternal plasma endothelin levels and fetal status in normal and preeclamptic pregnancies. *Gynecol Obst Invest* 1995; 39:88-92.

264. Frasch HF, Marshall C, Marshall BE. Endothelin-1 is elevated in monocrotaline pulmonary hypertension. *Am J Physiol* 1999; 276:L304-10.

265. Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Int Med* 1991; 114:464-9.

266. Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in lungs of patients with pulmonary hypertension. *N Engl J Med* 1993; 328:1732-9.

267. Franco-Cereceda A, Holm P. Selective or nonselective endothelin antagonists in porcine hypoxic pulmonary hypertension. *J Cardiovasc Pharmacol* 1998; 31 Suppl 1:s447-52.

268. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet* 2001; 358(9288):1119-23.

269. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med* 2002; 346:896-903.

270. Barst RJ, Rich S, Widlitz A, Horn EM, McLaughlin V, McFarlin J. Clinical efficacy of sitaxsentan, an endothelin-A receptor antagonist, in patients with pulmonary arterial hypertension: open-label pilot study. *Chest* 2002; 121:1860-8.

271. Vancheeswaran R, Magoulas T, Efrat G, Wheeler-Jones C, Olsen I, Penny R, Black CM. Circulating endothelin-1 levels in systemic sclerosis subsets - a marker of fibrosis or vascular dysfunction? *J Rheumatol* 1994; 21:1838-44.

272. Morelli S, Ferri C, Di Francesco L, et al. Plasma endothelin-1 levels in patients with systemic sclerosis: influence of pulmonary or systemic arterial hypertension. *Annal Rheum Dis* 1995; 54:730-4.

273. Odoux C, Crestani B, Lebrun G, et al. Endothelin-1 secretion by alveolar macrophages in systemic sclerosis. *Am J Resp Crit Care Med* 1997; 156:1429-35.

274. Kikuchi K, Kadono T, Sato S, Tamaki K, Takehara K. Impaired growth response to endothelin-1 in scleroderma fibroblasts. *Biochem Biophys Res Commun* 1995; 207:829-38.

275. Abraham DJ, Vancheeswaran R, Dashwood MR, et al. Increased levels of endothelin-1 and differential endothelin type A and B receptor expression in scleroderma-associated fibrotic lung disease. *Am J Pathol* 1997; 151:831-41.

276. Kramer BK, Ittner ME, Beyer ME, Hoffmeister HM, Riegger GA. Circulatory and myocardial effects of endothelin. *J Mol Med* 1997; 75:886-90.

277. Prasad MR. Endothelin stimulates degradation of phospholipids in isolated rat hearts. *Biochem Biophys Res Commun* 1991; 174:952-7.

278. Sakai S, Miyauchi T, Kobayashi M, Yamaguchi I, Goto K, Sugishta Y. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. *Nature* 1996; 384:353-5.

279. Sakai S, Miyauchi T, Sakurai T, et al. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats with congestive heart failure. Marked increase in endothelin-1 production in the failing heart. *Circulation* 1996; 93:1214-22.

280. Wei CM, Clavell AL, Burnett JCJ. Atrial and pulmonary endothelin mRNA is increased in a canine model of chronic low cardiac output. *Am J Physiol* 1997; 273:R838-44.

281. Tonnessen T, Lunde PK, Giaid A, Sejersted PM, Christensen G. Pulmonary and cardiac expression of preproendothelin-1 mRNA are increased in heart failure after myocardial infarction in rats. Localization of preproendothelin-1 mRNA and endothelin peptide. *Cardiovas Res* 1998; 39:633-43.

282. Iwanaga Y, Kihara Y, Hasegawa K, et al. Cardiac endothelin-1 plays a critical role in the functional deterioration of left ventricles during the transition from compensatory

hypertrophy to congestive heart failure in salt-sensitive hypertensive rats. *Circulation* 1998; 98:2065-73.

283. Teerlink JR, Breu V, Sprecher U, Clozel M, Clozel JP. Potent vasoconstriction mediated by endothelin ETB receptors in canine coronary arteries. *Circ Res* 1994; 74:105-14.

284. Cannan CR, Burnett JCJ, Lerman A. Enhanced coronary vasoconstriction to endothelin-B-receptor activation in experimental congestive heart failure. *Circulation* 1996; 93:646-51.

285. Love MP, Haynes WG, Gray GA, Webb DJ, McMurray JJV. Vasodilator effects of endothelin-converting enzyme inhibition and endothelin ETA receptor blockade in chronic heart failure patients treated with ACE inhibitors. *Circulation* 1996; 94:2131-7.

286. Kelso EJ, Geraghty RF, McDermott BJ, Trimble ER, Nicholls DP, Silke B. Mechanical effects of ET-1 in cardiomyocytes isolated from normal and heart-failed rabbits. *Mol Cell Biochem* 1996; 157:149-55.

287. Thomas PB, Liu EC, Webb ML, Mukherjee R, Hebbar L, Spinale FG. Exogenous effects and endogenous production of endothelin in cardiac myocytes: potential significance in heart failure. *Am J Physiol* 1996; 271:H2629-37.

288. Mulder P, Richard V, Derumeaux G, et al. Role of endogenous endothelin in chronic heart failure: effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodelling. *Circulation* 1997; 96:1976-82.

289. Shen Y-T, Buie P, Moussa T, Lynvh JL, Ma X-L. Chronic therapy with L-753,037, an $ET_{A/B}$ receptor antagonist, in conscious dogs during development of heart failure (abstract). *Circulation* 1998; 98:I-578.

290. Pacher R, Bergler-Klein J, Globits S, et al. Plasma big endothelin-1 concentrations in congestive heart failure patients with or without systemic hypertension. *Am J Cardiol* 1993; 71:1293-9.

291. Wei CM, Lerman A, Rodeheffer RJ, et al. Endothelin in human congestive heart failure. *Circulation* 1994; 89:1580-6.

292. Kiowski W, Sutsch G, Hunziker P, et al. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet* 1995; 346:732-6.

293. Sutsch G, Kiowski W, Yan X-W, et al. Short-term oral endothelin-receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. *Circulation* 1998; 98:2262-8.

294. Packer M, Caso A, Charlon V, al. e. Multicenter, double-blind, placebo-controlled study of long-term endothelin blockade with bosentan in chronic heart failure: results of the REACH-1 trial (abstract). *Circulation* 1998; 98:I-3.

295. Kalra PR, Moon JC, Coats AJ. Do results of the ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) study spell the end for non-selective endothelin antagonism in heart failure? *Int J Cardiol 2002* 2002; 85:195-7.

296. Torre-Amione G, Young JB, Colucci WS, et al. Hemodynamic and clinical effects of tezosentan, an intravenous dual endothelin receptor antagonist, in patients hospitalized for acute decompensated heart failure. *J Am Coll Cardiol* 2003; 42:140-7.

297. O'Connor CM, Gattis WA, Adams KFJ, et al. Tezosentan in patients with acute heart failure and acute coronary syndromes: results of the Randomized Intravenous TeZosentan Study (RITZ-4). *J Am Coll Cardiol* 2003; 41:1452-7.

298. Cotter G, Kaluski E, Stangl K, et al. The hemodynamic and neurohormonal effects of low doses of tezosentan (an endothelin A/B receptor antagonist) in patients with acute heart failure. *Eur J Heart Fail* 2004; 6:601-9.

299. Cowburn PJ, Cleland JGF, McArthur JD, MacLean MR, McMurray JJV, Dargie HJ. Short-term haemodynamic effects of BQ-123, a selective endothelin ET_A-receptor antagonist, in chronic heart failure. *Lancet* 1998; 352:201-2.

300. Givertz MM, Colucci WS, Gottlieb SS, et al. Acute ET_A receptor blockade reduces pulmonary vascular resistance in patients with chronic heart failure. *Circulation* 1998; 98:I-578.

301. Cowburn PJ, Cleland JGF, McDonagh TA, McArthur JD, MacLean MR, Dargie HJ. Adverse hemodynamic effects of a selective endothelin ET_B receptor antagonist in patients with chronic heart failure: reversal with a selective endothelin ET_A receptor antagonist. *Circulation* 1998; 98:I-718.

302. Luscher TF, Enseleit F, Pacher R, et al. Hemodynamic and neurohumoral effects of selective endothelin A (ET(A)) receptor blockade in chronic heart failure: the Heart Failure ET(A) Receptor Blockade Trial (HEAT). *Circulation* 2002; 106:2666-72.

303. Anand I, McMurray J, Cohn JN, et al. Long-term effects of darusentan on left-ventricular remodelling and clinical outcomes in the EndothelinA Receptor Antagonist Trial in Heart Failure (EARTH): randomised, double-blind, placebo-controlled trial. *Lancet* 2004; 364:327-54.

304. Kruger D, Sheikzadeh A, Giannitsis E, Stierle U. Cardiac release and kinetics of endothelin after severe short-lasting myocardial ischaemia. *J Am Coll Cardiol* 1997; 30:942-6.

305. Yasuda M, Kohno M, Tahara A, et al. Circulating immunoreactive endothelin in ischaemic heart disease. *Am Heart J* 1990; 119:801-6.

306. Toyo-oka T, Aizawa T, Suzuki N, et al. Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. *Circulation* 1991; 83:476-83.

307. Ray SG, McMurray JJ, Morton JJ, Dargie HJ. Circulating endothelin in acute ischaemic syndromes. *Br Heart J* 1992; 67:383-6.

308. Wieczorek I, Haynes WG, Webb DJ, Ludlam CA, Fox KAA. Raised plasma endothelin in unstable angina and non-Q wave myocardial infarction: relation to cardiovascular outcome. *Br Heart J* 1994; 72:436-41.

309. Omland T, Lie RT, Aakvaag A, Aarsland T, Dickstein K. Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction. *Circulation* 1994; 89:1573-9.

310. Richards AM, Webb DJ, Yandle TG, et al. Comparison of amino terminal pro-brain natriuretic peptide and big endothelin-1 as prognostic indicators after myocardial infarction. *J Am Coll Cardiol* 1999; 33:194A.

311. Awane-Igata Y, Ikeda S, Watanabe T. Inhibitory effects of TAK-044 on endothelin induced vasoconstriction in various canine arteries and porcine coronary arteries: a comparison with selective ETA and ETB receptor antagonists. *Br J Pharmacol* 1997; 120:516-22.

312. Wenzel RR, Fleisch M, Shaw S, et al. Haemodynamic and coronary effects of the endothelin antagonist bosentan in patients with coronary artery disease. *Circulation* 1998; 98:2235-40.

313. Grover GJ, Dzonczyk S, Parham CS. The endothelin-1 receptor antagonist BQ-123 reduces infarct size in a canine model of coronary occlusion and reperfusion. *Cardiovasc Res* 1993; 27:1613-8.

314. Watanabe T, Awane Y, Ikeda S, et al. Pharmacology of a non-selective ETA and ETB receptor antagonist, TAK 044 and the inhibition of myocardial infarct size in rats. *Br J Pharmacol* 1995; 114:949-54.

315. Zeiher AM, Gobel H, Schachinger V, Ihling C. Tissue endothelin-1 immunoreactivity in the active coronary atherosclerotic plaque. A clue to the mechanism of increased vasoreactivity of the culprit lesion in unstable angina. *Circulation* 1995; 91:941-7.

316. Kowala MC, Rose PM, Stein PD, et al. Selective blockade of the endothelin subtype A receptor decreases early atherosclerosis in hamsters fed cholesterol. *Am J Pathol* 1995; 146:819-26.

317. Barton M, Haudenschild CC, D'Uscio LV, Shaw S, Munter K, Luscher TF. Endothelin ET(A) receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. *Proc Nat Acad Sci* 1998; 95:14367-72.

318. Best PJM, McKenna CJ, Hasdai D, Holmes DRJ, Lerman A. Chronic endothelin receptor antagonism preserves coronary endothelial function in experimental hypercholesterolaemia. *Circulation* 1999; 99:1747-52.

319. Hirata Y, Takagi Y, Fukuda Y, Marumo F. Endothelin is a potent mitogen for rat vascular smooth muscle cells. *Atherosclerosis* 1989; 78:225-8.

320. Weissberg PL, Witchell C, Davenport AP, Hesketh TR, Metcalfe JC. The endothelin peptides ET-1, ET-2, ET-3 and sarafotoxin S6b are co-mitogenic with platelet-derived growth factor for vascular smooth muscle cells. *Atherosclerosis* 1990; 85:257-62.

321. Wang X, Douglas SA, Louden C, Vickery-Clarke LM, Feuerstein GZ, Ohlstein EH. Expression of endothelin-1, endothelin-3, endothelin-converting enzyme-1, and endothelin-A and endothelin-B receptor mRNAs after angioplasty induced neointimal formation in the rat. *Circ Res* 1996; 78:322-8.

322. Douglas SA, Louden C, Vickery-Clark LM, et al. A role for endogenous endothelin-1 in neointimal formation after rat carotid artery balloon angioplasty. Protective effects of the novel non-peptide endothelin receptor antagonist SB 209670. *Circ Res* 1994; 75:190-7.

323. Tahara A, Kohno M, Yanagai S, et al. Circulating immunoreactive endothelin in patients undergoing percutaneous transluminal coronary angioplasty. *Metabolism* 1991; 40:1235-7.

324. Sanmartin M, Fernandez-Ortiz A, Fantidis P, et al. Effects of bosentan on neointimal response following coronary angioplasty. *Eur J Clin Invest* 2003; 33:762-8.

325. Douglas SA, Vickery-Clarke LM, Louden C, Elliott JD, Ohlstein EH. Endothelin receptor subtypes in the pathogenesis of angioplasty-induced neointima formation in the rat: a comparison of selective ETA receptor antagonism and dual ETA/ETB receptor antagonism using BQ-123 and SB 209670. *J Cardiovasc Pharmacol* 1995; 26:S186-9.

326. Chandra S, Sarkar S, Elliott JD, Ohlstein EH. Application of magnetic resonance imaging for evaluation of the efficacy of SB 217242 in neointimal formation. *J Cardiovasc Pharmacol* 1998; 31:S317-9.

327. McKenna CJ, Burke SE, Opgenorth TJ, et al. Selective ET(A) receptor antagonism reduces neointimal hyperplasia in a porcine coronary stent model. *Circulation* 1998; 97:2551-6.

328. Douglas SA. Clinical development of endothelin receptor antagonists. *Trend Pharmacol Sci* 1997; 18:408-12.

329. Douglas SA, Nichols AJ, Feuerstein GZ, Elliott JD, Ohlstein EH. SB 209670 inhibits the arrhythmogenic actions of endothelin-1 in the anesthetized dog. *J Cardiovasc Pharmacol* 1998; 31 Suppl 1:S99-102.

330. Raschack M, Juchelka F, Rozek-Schaefer G. The Endothelin-A antagonist LU 135 252 supresses ischaemic extrasystoles and fibrillation in pigs and prevents hypoxic cellular decoupling. *J Cardiovasc Pharmacol* 1998; 31 Suppl 1:S145-8.

331. Garjani A, Wainwright CL, Zeitlin I, Wilson C, Slee S. Effects of endothelin-1 and the ET(A) receptor antagonist BQ-123, on ischaemic arrhythmias in anesthetized rats. *J Cardiovasc Pharmacol* 1995; 25:634-42.

332. Kaasjager KAH, Koomans HA, Rabelink TJ. Effectiveness of enalapril versus nifedipine to antagonise blood pressure and the renal response to endothelin in humans. *Hypertension* 1995; 25:620-5.

333. Webb ML, Dickinson KEJ, Delaney CL, Liu EC-K, Serafino R. The endothelin receptor antagonist, BQ-123, inhibits angiorensin II-induced contractions in rabit aorta. *Biochem Biophys Res Commun* 1992; 185:887-92.

334. Collier JG, Robinson BF, Vane JR. Reduction of the pressor effects of Angiotensin I in man by synthetic nonapeptide B.P.P.5a or SQ 20,881 which inhibits converting enzyme. *Lancet* 1973; i (7794):72-4.

335. Hollenberg NK, Williams GH, Burger B, Ishakawa I, Adams DF. Blockade and stimulation of renal, adrenal and vascular angiotensin II receptors with 1-sar, 8-ala angiotensin II in normal man. *J Clin Invest* 1976; 57:39-46.

336. Bramnert M, Hallengren B, Lecerof H, Werner R, Manhem P. Decreased blood pressure response to infused noradrenaline in normotensive as compared to hypertensive patients with primary hypothyroidism. *Clin Endocrinol* 1994; 40:317-21.

337. Biollaz J, Schelling JL, Jacot Des Combes B, et al. Enalapril maleate and a lysine analogue (MK-521) in normal volunteers; relationships between plasma drug levels and the renin angiotensin system. *Br J Clin Pharmacol* 1982; 14:363-8.

338. Davies RO, Gomez HJ, Irvin JD, Walker JF. An overview of the clinical pharmacology of enalapril. *British Journal of Pharmacology* 1984; 18:215S-229S.

339. Brunner DB, Desponds G, Biollaz J, et al. Effects of a new angiotensin converting enzyme inhibitor MK 421 and its lysine analogue on the components of the renin system in healthy subjects. *Br J Clin Pharmacol* 1981; 11:461-7.

340. MacGregor GA, Markandu ND, Bayliss J, Roulston JE, Squires M, Morton JJ. Non-sulphydryl-containing angiotensin-converting enzyme inhibitor (MK-421): Evidence for the role of renin system in normotensive subjects. *Br Med J* 1981; 283:401-3.

341. MacFadyen RJ, Elliot HL, Meredith PA, Reid JL. Haemodynamic and hormonal responses to oral enalapril in salt depleted normotensive man. *Br J Clin Pharmacol* 1993; 35:299-301.

342. Ribeiro W, Muscara MN, Martins AR, Moreno HJ, G.B. M, de Nucci G. Bioequivalence study of two enalapril maleate tablet formulations in healthy male volunteers. *Br J Clin Pharmacol* 1996; 50:399-405.

343. Sanchez RA, Marco E, Gilbert HB, Raffaele P, Giminez M, Moledo LI. Natriuretic effect and changes in renal haemodynamics induced by enalapril in essential hypertension. *Drugs* 1985; 30 Suppl 1:49-58.

344. Haynes WG, Noon JP, Walker BR, Webb DJ. Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens* 1993; 11:1375-80.

345. Olsen NV, Jensen NG, Hansen JM, Christensen NJ, Fogh-Andersen N, Kanstrup IL. Non-steroidal anti-inflammatory drugs and renal response to exercise: a comparison of indomethacin and nabumetone. *Clin Sci* 1999; 97:457-6.

346. Levinsky NG, Levy M. Clearance techniques. In: Orloff J, Berliner RW, eds. Handbook of Physiology. Vol. Section 8: Renal Physiology. Baltimore: Williams & Wilkins Company, 1973:103-117.

347. Hansen JM, Fogh-Andersen N, Christensen NJ, Strandgaard S. Cyclosporine-induced hypertension and decline in renal function in healthy volunteers. *J Hypertens* 1997; 15:319-26.

348. Benjamin N, Calver A, Collier J, Robinson B, Vallance P, Webb D. Measuring forearm bloodflow and interpreting the responses to drugs and mediators. *Hypertension* 1995; 25:918-23.

349. Wiinberg N, Walter-Larson S, Eriksen C, Nielsen PE. An evaluation of semi-automatic blood pressure manometers against intra-arterial blood pressure. *Journal of Ambulatory Monitoring* 1988; 1:303-9.

350. Goldstein DS, Cannon RO, Zimlichman R. Clinical evaluation of impedance cardiography. *Clin Physiol* 1986; 6:235-8.

351. Thomas SHL. Impedance cardiography using the Sramek-Bernstein method: accuracy and reproducibility at rest and during exercise. *Br J Clin Pharmacol* 1992; 34:467-76.

352. de Mey C, Belz CG. Pitfalls and limitations in the use of impedance cardiography. *Br Heart J* 1989; 61:128-9.

353. Rolinski B, Bonger SJ, Goebel FD. Determination of endothelin-1 immunoreactivity in plasma, cerebrospinal fluid and urine. *Res Exp Med* 1994; 194:9-24.

354. Morton JJ, Webb DJ. Measurement of plasma angiotensin II. *Clin Sci* 1985; 68:483-4.

355. Cody RJ, Haas GJ, Binkley PF, Capers Q, Kelley R. Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. *Circulation* 1992; 85:504-9.

356. Newby DE, Jalan R, Masumori S, Hayes PC, Boon NA, Webb DJ. Peripheral vascular tone in patients with cirrhosis: role of the renin-angiotensin and sympathetic nervous systems. *Cardiovasc Res* 1998; 38:221-8.

357. Haber E, Koerner T, Page LB, Kilman B, Purnode A. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocrinol Metab* 1969; 29:1349-55.

358. Kubasik N, Warren K, Sine HE. Evaluation of a new commercial radioassay kit for aldosterone using an iodinated tracer. *Clin Biochem* 1979; 12:59-61.

359. Holmquist B, Bunning P, Riordan JF. A continuous spectrophotometric method for the measurement of angiotensin-converting-enzyme in human serum. *Anal Biochem* 1979; 95:540-8.

360. Webb DJ. The pharmacology of human blood vessels in vivo. *J Vasc Res* 1995; 32:2-15.

361. Newby DE, Flint LL, Fox KA, Boon NA, Webb DJ. Reduced responsiveness to endothelin-1 in peripheral resistance vessels of patients with syndrome X. *J Am Coll Cardiol* 1998; 37:1585-90.

362. Fukuroda T, Fujikawa T, Ozakai S, Ishikawa K, Yano M, Nishikibe M. Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem Biophys Res Commun* 1994; 199:1461-5.

363. Ozaki S, Ohwaki K, Ihara M, Fukuroda T, Ishikawa K, Yano M. ETB-mediated regulation of extracellular levels of endothelin-1 in cultured human endothelial cells. *Biochem Biophys Res Commun* 1995; 209:483-9.

364. Dupuis J, Goresky CA, Fournier A. Pulmonary clearance of circulating endothelin-1 in dogs in vivo: Exclusive role of ET(B) receptors. *J Appl Physiol* 1996; 81:1510-5.

365. Benjamin N, Cockcroft JR, Collier JG, Dollery CT, Ritter JM, Webb DJ. Local inhibition of converting enzyme and vascular responses to angiotensin and bradykinin in the human forearm. *J Physiol (Lond)* 1989; 412:543-5.

366. Anonymous. Effects of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure.The SOLVD investigators. *New Engl J Med* 1991; 325:293-302.

367. Anonymous. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD investigators. *New Engl J Med* 1992; 327:685-91.

368. Neal B, MacMahon S, Chapman N. Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: results of prospectively designed overviews of randomised trials. Blood Pressure Lowering Treatment Trialists' Collaboration. *Lancet* 2000; 356:1942-3.

369. Herizi A, Jover B, Bouriquet N, Mimran A. Prevention of the cardiovascular and renal effects of angiotensin II by endothelin blockade. *Hypertension* 1998; 31:10-4.

370. d'Uscio LV, Moreau P, Shaw S, Takase H, Barton M, Luscher TF. Effects of chronic ET_A-receptor blockade in angiotensin II-induced hypertension. *Hypertension* 1997; 29:435-41.

371. Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M, Luscher TF. Angiotensin II increases tissue endothelin and induces vascular hypertrophy. Reversal by ET_A-receptor antagonist. *Circulation* 1997; 96:1593-7.

372. Barton M, Shaw S, d'Uscio LV, Moreau P, Luscher TF. Differential modulation of the renal and myocardial endothelin systems by angiotensin II in vivo. Effects of chronic selective ETA receptor blockade. *J Cardiovasc Pharmacol* 1998; 31 Suppl 1:S265-8.

373. Ortiz MC, Sanabria E, Manriquez MC, Romero JC, Juncos LA. Role of endothelin and isoprostanes in slow pressor response to angiotensin II. *Hypertension* 2001; 37:505-10.

374. Alexander BT, Cockrell KL, Rinewalt AN, Herrington JN, Granger JP. Enhanced renal expression of preproendothelin mRNA during chronic angiotensin II hypertension. *Am J Physiol* 2001; 280:R1388-92.

375. Gardiner SM, March JE, Kemp PA, Bennett T. Cardiovascular responses to angiotensins I and II in normotensive and hypertensive rats; effects of NO synthase inhibition or ET receptor antagonism. *Br J Pharmacol* 1999; 128:1795-1803.

376. Boemke W, Hocher B, Schleyer N, Krebs MO, Kaczmarczyk G. Hemodynamic, renal, and endocrine responses to acute ETA blockade at different ANG II plasma levels. *Am J Physiol* 2001; 280:R1322-31.

377. Balakrishnan SM, Wang HD, Gopalakrishnan V, Wilson TW, McNeill JR. Effect of an endothelin antagonist on hemodynamic responses to angiotensin II. *Hypertension* 1996; 28:806-9.

378. Heinemann A, Wachter CH, Peskar BA, Holzer P. Dilatation by angiotensin II of the rat femoral arterial bed in vivo via pressure/flow induced release of nitric oxide and prostaglandins. *Br J Pharmacol* 1997; 122:975-84.

379. Riggleman A, Harvey J, Baylis C. Endothelin mediates some of the renal actions of acutely administered angiotensin II. *Hypertension* 2001; 38:105-9.

380. Sung CP, Arleth AJ, Storer BL, Ohlstein EH. Angiotensin type 1 receptors mediate smooth muscle proliferation and endothelin biosynthesis in rat vascular smooth muscle. *J Pharmacol Exp Therapeut* 1994; 271:429-37.

381. Scott-Burden T, Resink TJ, Hahn AW, Vanhoutte PM. Induction of endothelin secretion by angiotensin II: effects on growth and synthetic activity of vascular smooth muscle cells. *J Cardiovasc Pharmacol* 1991; 17 Suppl 7:S96-100.

382. Ferri C, Desideri G, Baldoncini R, Bellini C, Valenti M, Santucci A, De Mattia G. Angiotensin II increases the release of endothelin-1 from human cultured endothelial cells but does not regulate its circulating levels. *Clin Sci* 1999; 96:261-70.

383. Casellas D, Bouriquet N, Herizi A. Bosentan prevents preglomerular alterations during angiotensin II hypertension. *Hypertension* 1997; 30:1613-20.

384. Kaddoura S, Firth JD, Boheler KR, Sugden PH, Poole-Wilson PA. Endothelin-1 is involved in norepinephrine-induced ventricular hypertrophy in vivo. Acute effects of bosentan, an orally active, mixed endothelin ETA and ETB receptor antagonist. *Circulation* 1996; 93:2068-79.

385. Yoshida K, Yasujima M, Kohzuki M, Kanazawa M, Yoshinaga K, Abe K. Endothelin-1 augments pressor response to angiotensin II infusion in rats. *Hypertension* 1992; 20:292-7.

386. Wenzel RR, Ruthemann J, Bruck H, Schafers RF, Michel MC, Philipp T. Endothelin-A receptor antagonist inhibits angiotensin II and noradrenaline in man. *Br J Clin Pharmacol* 2001; 52:151-7.

387. Benigni A, Remuzzi G. Endothelin antagonists. *Lancet* 1999; 353:133-8.

388. Love MP, Ferro CJ, Haynes WG, Plumpton C, Davenport AP, Webb DJ, McMurray JJ. Endothelin receptor antagonism in patients with chronic heart failure. *Cardiovasc Res* 2000; 47:166-72.

389. Schmidt RJ, Domico J, Samsell LS, et al. Indices of activity of the nitric oxide system in hemodialysis patients. *Am J Kidney Diss* 1999; 34:228-34.

390. Kato M, Roberts-Thomson P, Phillips BG, Haynes WG, Winnicki M, Accurso V, Somers VK. Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. *Circulation* 2000; 102:2607-10.

391. Paniagua OA, Bryant MB, Panza JA. Role of endothelial nitric oxide in shear stress-induced vasodilation of human microvasculature: diminished activity in hypertensive and hypercholesterolemic patients. *Circulation* 2001; 103:1752-8.

392. Tschope C, Schultheiss HP, Walther T. Multiple interactions between the renin-angiotensin and the kallikrein-kinin systems: role of ACE inhibition and AT1 receptor blockade. *J Cardiovasc Pharmacol* 2002; 39:478-87.

393. Berthold H, Munter K, Just A, Kirchheim HR, Ehmke H. Contribution of endothelin to renal vascular tone and autoregulation in the conscious dog. *Am J Physiol* 1999; 279:F417-24.

394. Kohan DE. Endothelins in the normal and diseased kidney. *Am J Kid Dis* 1997; 29:2-26.

395. Newby DE, Strachan FE, Webb DJ. Abnormal endothelin B receptor vasomotor responses in patients with Hirschprung's disease. *Q J Med* 2002; 95:159-63.

396. Goddard J, Webb DJ. Endothelin receptor antagonists: promising new agents in the management of cardiovascular disorders. *Drugs R & D* 1999; 2:1-12.

397. d'Uscio LV, Barton M, Shaw S, Luscher TF. Chronic ET(A) receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. *Cardiovasc Res* 2002; 53:487-95.

398. Tostes RC, Touyz RM, He G, Ammarguellat F, Schiffrin EL. Endothelin A receptor blockade decreases expression of growth factors and collagen and improves matrix metalloproteinase-2 activity in kidneys from stroke-prone spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 2002; 39:892-900.

399. Ammarguellat F, Larouche II, Schiffrin EL. Myocardial fibrosis in DOCA-Salt hypertensive Rats : effect of endothelin ET(A) receptor antagonism. *Circulation* 2001; 103:319-24.

400. Li JS, Turgeon A, Schiffrin EL. Effect of chronic treatment with two different ET(A) selective endothelin receptor antagonists on blood pressure and small artery structure of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. *Am J Hypertens* 1998; 11:554-62.

401. Rossi GP, Sacchetto A, Cesari M, Pessina AC. Interactions between the endothelin-1 and the renin-angiotensin-aldosterone system. *Cardiovasc Res* 1999; 43:300-7.

402. Kohno M, Horio T, Ikeda M, et al. Angiotensin II stimulates endothelin-1 secretion in cultured rat mesangial cells. *Kidney Int* 1992; 42:860-6.

403. Moroi M, Fukazawa M, Ishakawa M, Aikawa J, Namiki A, Yamaguchi T. Effect of endothelin on angiotensin converting enzyme activity in cultured vascular smooth muscle cells. *Gen Pharmacol* 1996; 27:463-5.

404. Bayerle-Eder M, Langenberger H, Pleiner J, et al. Endothelin ETA receptor-subtype specific antagonism does not mitigate the acute systemic or renal effects of exogenous angiotensin II in humans. *Eur J Clin Invest* 2002; 32:230-5.

405. Mortensen LH, Fink GD. Captopril prevents chronic hypertension produced by infusion of endothelin-1 in rats. *Hypertension* 1992; 19:676-80.

406. Elmarakby AA, Morsing P, Pollock DM. Enalapril attenuates endothelin-1-induced hypertension via increased kinin survival. *Am J Physiol* 2003; 284:H1899-903.

407. Amann K, Simonaviciene A, Medwedewa T, et al. Blood pressure-independent additive effects of pharmacologic blockade of the renin-angiotensin and endothelin systems on progression in a low-renin model of renal damage. *J Am Soc Nephrol* 2001; 12:2572-84.

408. Bauersachs J, Fraccarollo D, Schafer A, Ertl G. Angiotensin-converting enzyme inhibition and endothelin antagonism for endothelial dysfunction in heart failure: mono or combination therapy. *J Cardiovasc Pharmacol* 2002; 40:594-600.

409. Mulder P, Boujedaini H, Richard V, Henry JP, Renet S, Munter K, Thuillez C. Long-term survival and hemodynamics after endothelin-A receptor antagonism and angiotensin-converting enzyme inhibition in rats with chronic heart failure: monotherapy versus combination therapy. *Circulation* 2002; 106:1159-64.

410. Montanari A, Carra N, Perinotto P, Iori V, Fasoli E, Biggi A, Novarini A. Renal hemodynamic control by endothelin and nitric oxide under angiotensin II blockade in man. *Hypertension* 2002; 39:715-20.

411. Moridaira K, Morrissey J, Fitzgerald M, Guo G, McCracken R, Tolley T, Klahr S. ACE inhibition increases expression of the ETB receptor in kidneys of mice with unilateral obstruction. *Am J Physiol* 2003; 284:F209-17.

412. Elijovich F, Laffer CL, Schiffrin EL, Gavras H, Amador E. Endothelin-aldosterone interaction and proteinuria in low-renin hypertension. *J Hypertens* 2004; 22:573-82.

413. Gray G, Webb DJ. The endothelin system and its potential as a therapeutic target in cardiovascular disease. *Pharmacol Ther* 1996; 72:109-48.

414. Nielsen CB, Sorensen SS, Pedersen EB. Identical effects of indomethacin on renal function in healthy uninephrectomized subjects and in healthy control subjects. *Clin Sci* 1994; 86:715-21.

415. Catella-Lawson F, McAdam B, Morrison BW, et al. Effects of Specific Inhibition of Cyclooxygenase-2 on Sodium Balance, Hemodynamics, and Vasoactive Eicosanoids. *J Pharmacol Exp Ther* 1999; 289:735-41.

416. Taddei S, Vanhoutte PM. Endothelium-dependent contractions to endothelin in the rat aorta are mediated by thromboxane A2. *J Cardiovasc Pharmacol* 1993; 22 Suppl 8:S328-31.

417. Taddei S, Vanhoutte PM. Role of endothelium in endothelin-evoked contractions in the rat aorta. *Hypertension* 1993; 21:9-15.

418. Hercule HC, Oyekan AO. Role of NO and cytochrome P-450-derived eicosanoids in ET-1-induced changes in intrarenal hemodynamics in rats. *Am J Physiol* 2000; 279:R2132-41.

419. Trang AJ, Teoh H, Ward ME. Inhibition of prostanoid-mediated contraction to endothelin-1 after hypoxia in rat aorta. *Eur J Pharmacol* 2001; 423:57-61.

420. Newaz MA, Oyekan AO. Contribution of renal oxygenases to glycerol-induced acute renal failure in the rat. *J Cardiovasc Pharmacol* 2002; 39:834-41.

421. Oliver JJ, Rajapakse NW, Evans RG. Effects of indomethacin on responses of regional kidney perfusion to vasoactive agents in rabbits. *Clin Exp Pharmacol Physiol* 2002; 29:873-9.

422. Hoffman A, Abassi ZA, Brodsky S, Ramadan R, Winaver J. Mechanisms of big endothelin-1-induced diuresis and natriuresis: role of ET(B) receptors. *Hypertension* 2000; 35:732-9.

423. Lariviere R, Day R, Schiffrin EL. Increased expression of endothelin-1 gene in blood vessels of deoxycorticosterone acetate-salt hypertensive rats. *Hypertension* 1993; 21:916-20.

424. Barton M, d'Uscio LV, Shaw S, Meyer P, Moreau P, Luscher TF. ET(A) receptor blockade prevents increased tissue endothelin-1, vascular hypertrophy, and endothelial dysfunction in salt-sensitive hypertension. *Hypertension* 1998; 31:499-504.

425. Orth SR, Esslinger JP, Amann K, Schwarz U, Raschack M, Ritz E. Nephroprotection of an ET(A)-receptor blocker (LU 135252) in salt-loaded uninephrectomized stroke-prone spontaneously hypertensive rats. *Hypertension* 1998; 31:955-1001.

426. Barton M, Vos I, Shaw S, et al. Dysfunctional renal nitric oxide synthase as a determinant of salt-sensitive hypertension: mechanisms of renal artery endothelial dysfunction and role of endothelin for vascular hypertrophy and glomerulosclerosis. *J Am Soc Nephrol* 2000; 11:835-45.

427. Ferri C, Bellini C, Desideri G, Giuliani E, De Siati L, Cicogna S, Santucci A. Clustering of endothelial markers of vascular damage in human salt-sensitive hypertension: influence of dietary sodium load and depletion. *Hypertension* 1998; 32:862-8.

428. Ferri C, Bellini C, Desideri G, Mazzocchi C, De Siati L, Santucci A. Elevated plasma and urinary endothelin-I levels in human salt-sensitive hypertension. *Clin Sci* 1997; 93:35-41.

429. Bech JN, Nielsen CB, Ivarsen P, Jensen KT, Pedersen EB. Dietary sodium affects systemic and renal haemodynamic responses to NO inhibition in healthy humans. *Am J Physiol* 1998; 274:F914-23.

430. Barba G, Vallance PJ, Strazzullo P, MacAllister RJ. Effects of sodium intake on the pressor and renal responses to nitric oxide synthesis inhibition in normotensive individuals with different sodium sensitivity. *J Hypertens* 2000; 18:615-21.

431. Chen PY, Sanders PW. L-arginine abrogates salt-sensitive hpertension in Dahl/Rapp rats. *J Clin Invest* 1991; 88:1559-67.

432. Matsuoka H, Itoh S, Kimoto M, et al. Asymmetrical dimethyarginine, an endogenous nitric oxide synthase inhibitor, in expeimental hypertension. *Hypertension* 1997; 29:242-7.

433. Pollock DM, Pollock JS. Evidence for endothelin involvement in the response to high salt. *Am J Physiol* 2001; 281:F144-50.

434. Matsumura Y, Kuro T, Kobayashi Y, et al. Exaggerated vascular and renal pathology in endothelin-B receptor-deficient rats with deoxycorticosterone acetate-salt hypertension. *Circulation* 2000; 102:2765-73.

435. Pollock DM, Allcock GH, Krishnan A, Dayton BD, Pollock JS. Upregulation of endothelin B receptors in kidneys of DOCA-salt hypertensive rats. *Am J Physiol* 2000; 278:F279-86.

436. Serneri GGN, Modesti PA, Cecioni I, et al. Plasma endothelin and renal endothelin are two distinct systems involved in volume homeostasis. *Am J Physiol* 1995; 268:H1829-37.

437. Masereeuw R, Terlouw SA, van Aubel RA, Russke FG, Miller DS. Endothelin B receptor mediated regulation of ATP-driven drug secretion in renal proximal tubule. *Mol Phamacol* 2000; 57:59-67.

438. Schwarz A, Godes M, Thone-Reineke C, Theuring F, Bauer C, Neumayer HH, Hocher B. Tissue-dependent expression of matrix proteins in human endothelin-1 transgenic mice. *Clin Sci* 2002; 103 Suppl 48:39S-43S.

439. Anonymous. Cyclosporin in cadaveric renal transplantation: one-year follow-up of a multicentre trial. *Lancet* 1983; 2:986-9.

440. Anonymous. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. *New Engl J Med* 1983; 309:809-15.

441. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: a new immunosuppressive agent for organ transplantation. *Annal Int Med* 1984; 202:667-82.

442. Loughran TPJ, Deeg HJ, Dahlberg S, Kennedy MS, Storb R, Thomas ED. Incidence of hypertension after marrow transplantation among 112 patients randomised to either cyclosporine or methotrexate as graft-versus-host disease prophylaxis. *Br J Haematol* 1985; 59:547-53.

443. Olivari MT, Antolick A, Ring WS. Arterial hypertension in heart transplant recipients treated with triple-drug immunosuppressive therapy. *J Heart Transplant* 1989; 8:34-9.

444. Stein CM, He H, Pincus T, Wood AJ. Cyclosporine impairs vasodilation without increased sympathetic activity in humans. *Hypertension* 1995; 26:705-10.

445. Lamb FS, Webb RC. Cyclosporine augments reactivity of isolated blood vessels. *Life Sci* 1987; 40:2571-8.

446. Scherrer U, Vissing SF, Morgan BJ, et al. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *New Engl J Med* 1990; 323:693-9.

447. Morgan BJ, Lyson T, Scherrer V, Victor RG. Cyclosporine causes sympathetically mediated elevations on arterial pressure in rats. *Hypertension* 1991; 18:458-66.

448. Curtis JJ, Luke RG, Jones P, Diethelm AG. Hypertension in cyclosporine-treated renal transplant recipients is sodium dependent. *Am J Med* 1988; 85:134-8.

449. Whitworth JA, Mills EH, Coghlan JP, et al. The haemodynamic effects of cyclosporin A in sheep. *Clin Exp Pharmacol Physiol* 1987; 14:573-80.

450. Kishimoto T, Tsujino T, Nakatani T, et al. Effect of ciclosporin on cardiac output and regional blood flow in rats: ciclosporin-induced nephropathy and its prevention with calcium channel blocker. *Nephron* 1992; 61:204-10.

451. Ventura HO, Lavie CJ, Messerli FH, Valentino V, Smart FW, Stapleton DD, Ochsner JL. Cardiovascular adaptation to cyclosporine-induced hypertension. *J Human Hypertens* 1994; 8:233-7.

452. Navarro JF, Mora C, Marcen R, et al. Acute cardiovascular effects of intravenous cyclosporine. *Int Urol Nephrol* 1996; 28:575-81.

453. Perico N, Ruggenenti P, Gaspari F, et al. Daily renal hypoperfusion induced by cyclosporine in patients with renal transplantation. *Transplantation* 1992; 54:56-60.

454. Ruggenenti P, Perico N, Mosconi L, al e. Calcium channel blockers protect transplant patients from cyclosporine-induced daily renal hypoperfusion. *Kidney Int* 1993; 46:706-11.

455. Hansen JM, Hoy CE, Strandgaard S. Fish oil and Cyclosporin A-induced renal hypoperfusion in kidney transplanted patients. *Nephrol Dial Transplant* 1995; 10:1745-50.

456. Pei Y, al e. Sustained vasoconstriction associated with daily cyclosporin dose in heart and lung transplant recipients: Potential pathophysiologic role of endothelin. *J Lab Clin Med* 1995; 125:113-9.

457. Conte G, Dal Canton A, Sabbatini M, et al. Acute cyclosporine renal dysfunction reversed by dopamine infusion in healthy subjects. *Kidney Int* 1989; 36:1086-92.

458. Fuiano G, Conte G, Sepe V, et al. Acute effects of ciclosporin on renal haemodynamics and urinary protein excretion in patients with the nephrotic syndrome. *Nephron* 1991; 59:369-74.

459. De Nicola L, Libetta C, Memoli B, et al. Acute renal and immunological effects of low-dose cyclosporin in humans. *Nephrol Dial Transplant* 1995; 10:1739-44.

460. Weir MR, Klassen DK, Shen SY, Sullivan D, Buddemeyer EU, Handwerger BS. Acute effects of intravenous cyclosporine on blood pressure, renal haemodynamics, and urine prostaglandin production of healthy humans. *Transplantation* 1990; 49:41-7.

461. Sturrock NDC, Lang CC, Struthers AD. Cyclosporin-induced hypertension preceeds renal dysfunction and sodium retention in man. *J Hypertens* 1993; 11:1209-16.

462. Tegzess AM. Prospective serial renal function studies in patients with non-renal disease treated with cyclosporin A. *Transplant Proceed* 1990; 20:530-3.

463. Balletta M, al e. Effect of a 2 month treatment with low doses of cyclosporin on renal function. *Nephrol Dial Transplant* 1991; 6:324-9.

464. Sturrock NDC, Lang CC, Baylis PH, Struthers A.D. Sequential effects of cyclosporine therapy on blood pressure, renal function and neurohormones. *Kidney Int* 1994; 45:1203-10.

465. Sturrock ND, Lang CC, MacFarlane LJ, Dockrell ME, Ryan M, Webb DJ, Struthers AD. Serial changes in blood pressure, renal function, endothelin and lipoprotein (a) during the first 9 days of cyclosporin therapy in males. *J Hypertens* 1995; 13:667-73.

466. Stroes ES, Luscher TF, de Groot FG, Koomans HA, Rabelink TJ. Cyclosporin A increases nitric oxide activity in vivo. *Hypertension* 1997; 29:570-5.

467. Morris ST, McMurray JJ, Rodger RS, Farmer R, Jardine AG. Endothelial dysfunction in renal transplant recipients maintained on cyclosporine. *Kidney Int* 2000; 57:1100-6.

468. Oriji GK, Keiser HR. Role of nitric oxide in cyclosporin A-induced hypertension. *Hypertension* 1998; 32:849-55.

469. Vaziri ND, Ni Z, Zhang YP, Ruzics EP, Maleki P, Y. D. Depressed renal and vascular nitric oxide synthase expression in cyclosporine-induced hypertension. *Kidney Int* 1998; 54:482-91.

470. Morris ST, McMurray JJ, Spiers A, Jardine AG. Impaired endothelial function in isolated human uremic resistance arteries. *Kidney Int* 2001; 60:1077-82.

471. Cardillo C, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with type II diabetes mellitus. *Circulation* 2002; 106:1783-7.

472. Love MP, Haynes WG, Webb DJ, McMurray JJ. Venous endothelin receptor function in patients with chronic heart failure. *Clin Sci* 2000; 98:65-70.

473. Bohm F, Ahlborg G, Johansson BL, Hansson LO, Pernow J. Combined endothelin receptor blockade evokes enhanced vasodilatation in patients with atherosclerosis. *Arterioscler Thromb Vasc Biol* 2002; 22:674-9.

474. Evans RG, Madden AC, Oliver JJ, Lewis TV. Effects of ET(A) - and ET(B)-receptor antagonists on regional kidney blood flow, and responses to intravenous endothelin-1, in anaesthetized rabbits. *J Hypertens* 2001; 19:1789-99.

475. Ruggenenti P, Perna A, Remuzzi G, Nefrologia. GIdSEi. ACE inhibitors to prevent end-stage renal disease: when to start and why possibly never to stop: a post hoc analysis of the REIN trial results. Ramipril Efficacy in Nephropathy. *J Am Soc Nephrol* 2001; 12:2832-7.

476. Peterson JC, Adler S, Burkart JM, et al. Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. *Ann Intern Med* 1995; 123:754-62.

477. Ruilope LM, van Veldhuisen DJ, Ritz E, Luscher TF. Renal function: the Cinderella of cardiovascular risk profile. *J Am Coll Cardiol* 2001; 38:1782-7.

Publications arising from work presented in this thesis

Goddard J, Webb DJ. Endothelin receptor antagonists: promising new agents in the management of cardiovascular disorders. **Drugs in R&D** 1999;2:1-12.

Goddard J, Webb DJ. Plasma endothelin concentrations in hypertension. **J Cardiovasc Pharmacol** 2000;35 Suppl. 1:S25-31.

Spratt JCS, **Goddard J,** Patel N, Strachan FE, Rankin AJ, Webb DJ. Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men. **Br J Pharmacol** 2001;134:648-54

Goddard J, Webb DJ. Endothelin antagonists and hypertension: a question of dose? **Hypertension** 2002; 40(3):e1-2

Goddard J, Johnston NR, Hand MF, Cumming, AD, Rabelink TJ, Rankin AJ, Webb DJ. Endothelin-A receptor antagonism reduces blood pressure and proteinuria in patients with chronic renal failure: a comparison of selective and non-selective ET receptor blockade. **Circulation** 2004;109:1186-93

Goddard J, Eckhart C, Johnston NR, Cumming, AD, Rankin AJ, Webb DJ. Endothelin-A receptor antagonism is synergistic with ACE inhibition in healthy volunteers via an ETB receptor mediated, NO dependent mechanism. **J Am Soc Nephrol** 2004;15:2601-10

Drugs R&D 1999 Jul; 2 (1): 1-12
1174-5886/99/0007-0001/$06.00/0

Endothelin Receptor Antagonists

Promising New Agents in the Management of Cardiovascular Disorders

Jane Goddard and *David J. Webb*

Clinical Pharmacology Unit & Research Centre, Department of Medical Sciences, University of Edinburgh, Scotland

Abstract

Since its discovery in 1988, endothelin (ET) has been widely implicated in the pathophysiology of cardiovascular disease. ET antagonists have favourable effects in experimental models of these conditions and have proved useful in elucidating the role of the ET system. Orally acting ET antagonists appear very promising in clinical trials, particularly in patients with chronic heart failure and hypertension, but more information on the roles of the ET receptor subtypes in health and disease is required so that an informed choice can be made between the use of endothelin-A (ET-A) receptor–selective and nonselective receptor antagonists.

The endothelins (ET) are a family of 21 amino acid peptides with powerful vasoconstrictor and pressor properties that were first described by Yanagisawa et al. in 1988.[1] Three different isopeptides, endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3), have so far been identified, each with distinct genes and tissue distributions.[1-3] Of the 3 peptides, ET-1 is the major endothelial isoform and is therefore likely to be the most important in the regulation of cardiovascular function. Therefore, this article, focuses primarily on ET-1. Its main site of production is the vascular endothelial cell but it is also produced by other cell types, including renal tubular and mesangial cells, vascular smooth muscle cells and epicardial cells.[4-6] In addition to its vasoconstrictor properties, ET-1 functions as a mitogen, modulates other hormone systems and influences ion and fluid transport in the gut and kidney.[7,8]

Regulation of the production of ET-1 is thought to be at the level of gene transcription. Enhanced gene transcription occurs in response to a wide range of stimuli including other vasoactive hormones [such as angiotensin II, adrenaline (epinephrine) and vasopressin], cytokines (such as interleukin-1 and endotoxin), and hypoxia, glucose and oxidised low density lipoprotein (LDL). In contrast, prostacyclin, nitric oxide (NO) and the natriuretic peptides all inhibit transcription of endothelin genes. The gene product is prepro-ET-1, a protein with 212 amino acids which is cleaved in stages to yield the largely inactive big ET-1, a 38-amino-acid precursor peptide. Endothelin converting enzyme (ECE), a metalloproteinase, then splits big ET-1 into the active mediator, ET-1, and its C terminal fragment[9] (fig. 1).

Two ET receptors have been identified and cloned: endothelin-A (ET-A) receptors have a higher affinity for ET-1 than either ET-2 or ET-3, whereas endothelin-B (ET-B) receptors have equal affinity for the 3 ET peptides.[10-12] Within the circulation, ET-A receptors are found on vascular smooth muscle cells and their activation results in vasoconstriction. ET-B receptors are also found on vascular smooth muscle cells, where they mediate vasoconstriction. However, ET-B receptors are more abundant on the vascular endothelium where their

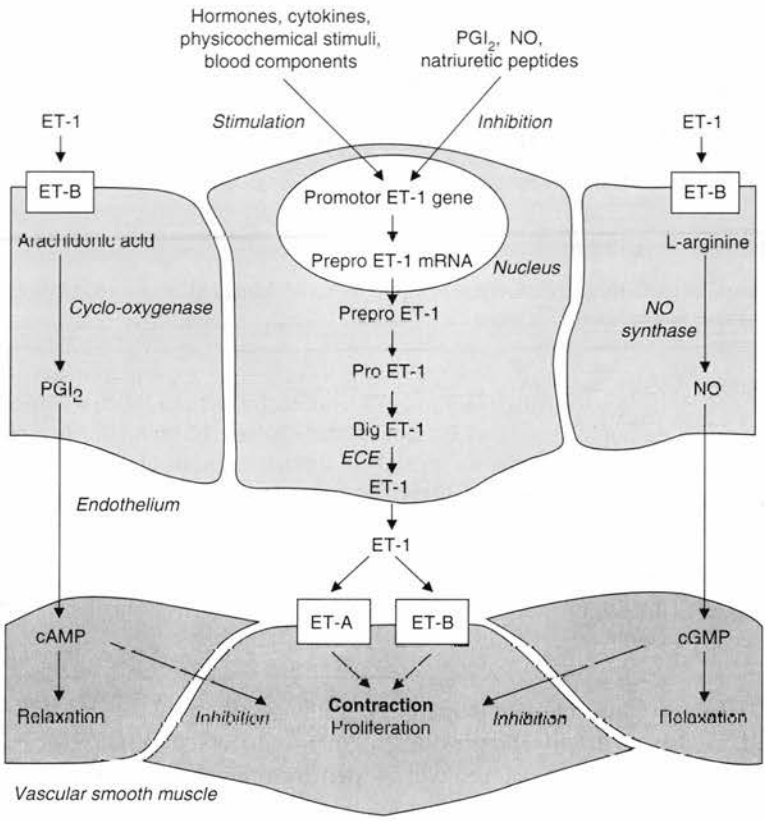


Fig. 1. The endothelin (ET) pathway: stimuli for ET production, pathway for generation and major effects mediated by ET-A and ET-B receptors in blood vessels. ET-1 is generated by endothelial cells and secreted abluminally to act on ET receptors on vascular smooth muscle cells promoting contraction. ET-1 can initiate relaxation via stimulation of ET-B receptors on the vascular endothelium promoting the generation of NO from L-arginine and PGI_2 from arachidonic acid. **cAMP** = cyclic adenosine monophosphate; **cGMP** = cyclic guanosine monophosphate; **ECE** = endothelin converting enzyme; **ET-A** = endothelin-A receptor; **ET-B** = endothelin-B receptor; **ET-1** = endothelin-1; **PGI_2** = prostacyclin; **mRNA** = messenger RNA; **NO** = nitric oxide.

activation results in vasodilatation mediated by NO and prostacyclin. In the human kidney, ET-A receptors are localised to vascular smooth muscle, notably in the glomeruli, vasa recta and arcuate arteries. ET-B receptors are more numerous (ET-B to ET-A ratio 2 : 1) and more widespread with a high concentration in the collecting system.[13] The distribution of these receptors within the kidney suggests a vasoactive role for ET-A receptors and a role in sodium and water handling for ET-B receptors.

Signal transduction is complex and reviewed elsewhere.[9] ET-1 is removed in the renal, splanchnic and pulmonary circulations, probably by ET-B receptor binding and internalisation and also enzymatic degradation by neutral endopeptidases.[14]

1. Endothelin Receptor Antagonists

Compounds that block the ET system have been used to define the role of the endogenous ET system and confirm its importance in cardiovascular function and dysfunction. Attention is now focusing increasingly on drugs that may have a role in clinical medicine. Though ECE inhibitors and monoclonal antibodies have been used experimentally,

and the former may be a therapeutic option in the future, most clinical work to date has been done with ET receptor antagonists.

ET receptor antagonists can be divided into orally active compounds and parenteral preparations. In certain situations an intravenous preparation will be preferred, notably to counter acute, isolated insults (e.g. cerebral ischaemia following subarachnoid haemorrhage or stroke, unstable angina, acute myocardial infarction, acute renal failure or postoperative pulmonary hypertension). However, many of the anticipated indications for ET receptor antagonists, including hypertension, chronic heart failure and coronary restenosis, will require chronic treatment for which oral agents are best suited (table I).

ET receptor antagonists can be further subdivided into nonselective ET receptor antagonists and ET-A–selective receptor antagonists. ET-A selectivity generally refers to compounds with ≥1000-fold affinity for the ET-A over the ET-B receptor. It is important to appreciate that so-called 'nonselective' antagonists are still generally selective for the ET-A over the ET-B receptor (table I). More work is needed to better delineate the respective roles of the ET-A and ET-B receptors in normal physiology and, more importantly, in pathophysiology before conclusions can be drawn regarding the relative benefit of nonselective versus selective ET-A receptor blockade in a given clinical situation.

A series of peptide antagonists that bind to, but do not activate ET receptors have been developed through the modification of ET peptides. Cyclic peptides were designed to mimic the hairpin loop of ET. For example, BQ 123 is a cyclic pentapeptide and ET-A–selective receptor antagonist that was developed from BE 18257B (a natural product of *Streptomyces misakiensis*). TAK 044 is a cyclic hexapeptide with nonselective ET receptor antagonist properties. A series of linear peptides with ET-B–selective receptor antagonist properties have also been developed (e.g. BQ 788). These peptides are broken down in the gut and, therefore, must be given parenterally after which they have relatively short half-lives. Experimentally, the half-life of BQ 123 can be increased by side-chain modifications that reduce hepatic extraction,[16] but the clinical utility of these compounds is limited by the lack of oral analogues.

A series of nonpeptide ET receptor antagonists with greater bioavailability than the peptides have been developed through the use of screening libraries and molecular modelling (table I). Bosentan (RO 470203), is a sulfonamide with nonselective receptor blocking activity that has the benefit of being available as both an oral and intravenous preparation. This will allow treatment to be rapidly initiated with the intravenous preparation in acute situations (e.g. exacerbations of heart failure) and continued thereafter with the oral formulation. SB 209670, a carboxylic acid derivative, is a more potent nonselective receptor antagonist than bosentan, but it is only available as an intravenous preparation. Enrasentan (SB 217242) is an analogue of SB 209670 with improved bioavailability that is currently in development as an oral drug.[17]

Orally active and selective ET-A receptor antagonists are being developed. ABT 627 is a carboxylic acid derivative with 2000-fold greater selectivity for the ET-A versus the ET-B receptor and an elimination half-life ($t_{½\beta}$) of 24 hours in humans[18] making it an attractive choice for situations requiring long term treatment (e.g. prevention of coronary restenosis). Minor structural modifications to ABT 627 yield highly selective ET-B receptor antagonists.[18] TBC 11251 and TA 0201 are sulfonamide derivatives with high bioavailability and ET-A receptor selectivity.[19] TA 0201 has a $t_{½\beta}$ of only 0.9 hours, but it is converted to an active metabolite with demonstrable inhibitory effect 8 hours after oral administration in rats.[20]

An interesting agent in preclinical development is L 747072, an angiotensin receptor antagonist discovered to have nonselective ET receptor antagonist activity, providing the potential for a dual therapeutic approach to the treatment of hypertension and other cardiovascular conditions.[17]

Table I. Endothelin receptor antagonists in development[a]

Name	Route	ET-A selectivity[b]	Phase	Anticipated indication	Structure	Manufacturer (co-developer)
Nonselective endothelin receptor antagonists						
Bosentan (RO 470203)	PO + IV	×20	III	Heart Failure	Sulfonamide	Roche
			II	Hypertension		
			Preclinical	Diabetic neuropathies, inflammatory bowel disease, transplant rejection		
				Ischaemia/reperfusion injury		
			Unknown	Ischaemic heart disease		
Enrasentan (SB 217242)	PO	×110	II	Pulmonary hypertension	Carboxylic acid derivative	SmithKline Beecham
			I	Heart Failure		
			Preclinical	Coronary restenosis, renal failure, stroke		
J 104132 (L 753037)	PO	×5	I	Hypertension	Carboxylic acid derivative	Merck & Co.
			Preclinical	Heart failure		
TAK 044	IV	×18	II	Myocardial infarction, renal failure, subarachnoid haemorrhage	Cyclic hexapeptide	Takeda
			I	Hypertension		
			Preclinical	Heart failure		
RO 610612	IV	NK	II	Cardiovascular disease, heart failure, hypertension	NK	Roche (Actelion)
			Preclinical	Reperfusion injury		
PD 145065	IV	×4	Preclinical	Hypertension, renal failure	Cyclic heptapeptide	Parke-Davis
			Unknown	Coronary disease		
ET-A–selective receptor antagonists						
TBC 11251 (IPI 1040)	PO + IV	×7000	II	Heart failure	Sulfonamide	Texas Biotechnology (LG Chem)
			Preclinical	Pulmonary hypertension		
LU 135252 (HMR 4005)	PO +IV	×130	II	Heart failure	Propionic acid derivative	Knoll (Hoechst Marion Roussel)
			Preclinical	Arrhythmias		
			Unknown	Hypertension		
ABT 627 (A 147627, ABT 147627)	PO	×2000	I	Coronary restenosis	Carboxylic acid derivative	Abbott
TA 0201 (T 0201)	PO	×2700	I	Heart failure	Sulfonamide	Tanabe Seiyaku
			Preclinical	Pulmonary hypertension, reperfusion injury		
ZD 1611	PO	×1000	I	Pulmonary hypertension	Propanoic acid derivative	AstraZeneca
			Preclinical	Heart failure, hypertension, chronic obstructive pulmonary disease		
BMS 182874	PO	×1000	Preclinical	Coronary disorders, hypertension	Sulfonamide	Bristol-Myers Squibb
RO 611790 (VML 588)	IV	×1000	I	Renal failure, subarachnoid haemorrhage	Sulfonamide	Roche (Vanguard Medica)
S 0139	IV	NK	I	Cerebral vasospasm, neuroprotection	Myriceric acid derivative	Shionogi
BQ 123	IV	×2500	Preclinical	Arrhythmias, cerebral ischaemia, diabetic complications, hypertension, ischaemic heart disease, stress ulcer, peripheral vascular disease	Cyclic pentapeptide	Banyu
			Unknown	Heart failure		
PD 147953 (FR 139317)	IV	×7000	Preclinical	Heart failure, diabetic complications, hypertension, ischaemic heart disease, renal failure	Linear tripeptide	Parke-Davis (Fujisawa)
			Unknown	Coronary disease		

a Drugs are listed (oral then IV) in order of the highest reported phase of development.

b Based on binding and functional assays.[14,15]

ET-A = endothelin-A; **IV** = intravenous; **NK** = not known; **PO** = oral.

2. Endothelin and the Cardiovascular System

The importance of ET-1 in cardiovascular function is suggested by the actions of exogenous ET-1, the administration of which is associated with an increase in blood pressure and peripheral vascular resistance.[21,22] The direct effects of ET-1 on the heart are probably species specific but a positive inotropic response to ET-1 is seen in isolated cardiac tissue from rats and humans,[23,24] and in *in vivo* models where coronary vasoconstriction is countered with a vasodilator.[25] The integrated haemodynamic response is, however, much more complicated. Increased peripheral vascular resistance and blood pressure produce reflex reductions in heart rate and cardiac output, and coronary vasoconstriction can cause myocardial ischaemia and cardiodepression. The effectiveness of receptor antagonists should clearly be judged on their integrated effects, as well as local effects on target organs or tissues.

In addition to its haemodynamic actions, exogenous ET-1 is mitogenic *in vitro*, thus causing cell proliferation and vascular remodelling, and caused renal sodium retention,[26] features of congestive heart failure (CHF) and hypertension.

3. Coronary Artery Disease

3.1 Ischaemia and Myocardial Infarction

Inducing coronary ischaemia in patients with coronary artery disease (CAD) results in a significant release of ET-1 of cardiac origin.[27] Plasma ET-1 concentrations are high in CAD, particularly in patients with myocardial infarction or coronary vasospasm,[28-31] and elevations of plasma ET-1 and big ET-1 in unstable angina and after myocardial infarction are correlated with a worse prognosis[32,33] suggesting that an activated ET system may contribute to a poor outcome.

Activation of ET-A and -B receptors in the coronary circulation contribute to vasoconstriction[34,35] and in isolated porcine and canine coronary arteries, TAK 044 inhibits ET-1–induced coronary vasoconstriction to a greater extent than BQ 123.[35] These data suggest that nonselective receptor blockade may be more effective than selective. In patients with CAD, acute intravenous administration of the nonselective antagonist bosentan increased the diameter of coronary epithelial arteries; an effect that was not augmented by administration of intravenous nitrates. This effect was, however, only clear in normal or mildly diseased arteries and was correlated inversely with plasma LDL-cholesterol levels.[36]

Both ET-A–selective (BQ 123) and nonselective (TAK 044) ET receptor antagonists have been shown to reduce infarct size in coronary ligation models.[37,38] Though these animal studies are promising, studies in humans with myocardial infarction have not been published.

3.2 Atherosclerosis

Plasma ET-1 concentrations are elevated in patients with atherosclerosis[39] and increased ET-1 levels has been demonstrated in atherosclerotic lesions[40] and throughout the vessel walls of patients with CAD[6] suggesting up-regulation of the ET system in atherosclerosis.

In hypercholesterolaemic hamsters, ET-A receptor inhibition decreases the progression of atherosclerosis mainly by reducing the number and size of macrophage-foam cells.[41] In addition, apolipoprotein E-deficient mice, which are genetically predisposed to severe atheroma and endothelial dysfunction, are protected from atherosclerosis when treated with ET-A receptor antagonists and show an increase in NO-mediated endothelium-dependent relaxation.[42] Similarly, hypercholesterolaemic pigs treated for a prolonged period with both selective and nonselective ET receptor antagonists have improved coronary vascular function.[43] In view of these results, it is possible that the failure of bosentan to significantly dilate severely diseased coronary arteries in humans[36] may relate to the greater degree of endothelial dysfunction in these arteries, and that this may respond to longer term administration of the drug.

3.3 Postangioplasty Restenosis

ET-1 has a mitogenic effect on vascular smooth muscle cells *in vitro*.[44,45] In animal studies, expression of messenger RNA (mRNA) for all of the components of the ET system is increased at balloon angioplasty sites,[46] and intra-arterial administration of ET-1 after angioplasty increases neointima formation.[47] In humans, an increase in plasma ET-1 concentration proportional to the duration and pressure of the balloon inflation is seen distal to the angioplasty site.[48,49] Nonselective ET receptor antagonism with SB 209670 or its analogue enrasentan has reduced neointima formation after endothelial injury by balloon angioplasty, whereas selective ET-A receptor antagonism with BQ 123 has been ineffective.[47,50,51] However, highly selective ET-A receptor blockade with the potent oral agent ABT 627 has been shown to reduce restenosis, indicating that combined receptor antagonism may not be required to achieve this effect.[52] Thus, prevention of restenosis with ET antagonists seems possible, although many other potentially useful agents have failed to fulfil their promise in this condition.

3.4 Arrhythmias

In animals, both combined ET receptor antagonism with SB 209670 and selective ET-A receptor antagonism with LU 135252 reduce the incidence of arrhythmias induced by ischaemia or exogenous ET-1.[15,53,54] Of note, however, while low doses (10 μg/kg/min) of BQ 123 reduced the frequency of ischaemic arrhythmias in rats, higher doses (100 μg/kg/min) increased mortality rates because of an increase in refractory ventricular fibrillation.[55]

4. Congestive Heart Failure

There is considerable evidence that ET-1 plays a role in CHF. ET-1 causes hypertrophy of cardiac myocytes[56] and has a direct toxic effect on these cells.[57] In animal models of CHF, increased cardiac and pulmonary ET-1 synthesis has been documented.[58-61] In a Dahl salt-sensitive, hypertensive rat model, the initial compensated left ventricular hypertrophy (LVH) was not associated with an increase in cardiac ET-1 levels, but a 5-fold increase was noted after the development of left ventricular (LV) dilatation and global hypokinesis.[62] ET-A receptors are more numerous than ET-B receptors in both the normal and failing heart, but ET-A receptors may be up-regulated in the failing heart suggesting that they may be the major therapeutic target.[24,58] As with CAD, however, some evidence points to the enhancement of vascular smooth muscle ET-B mediated vasoconstriction in CHF.[63-65] Also, although the contractility of myocytes obtained from animals and humans with CHF is attenuated in response to exogenous ET-1,[24,66,67] there is some evidence that endogenous ET-1 is involved in the maintenance of cardiac function in CHF.[58] Thus, caution is necessary in contemplating blockade of the ET system in patients with heart failure.

Animal models of CHF have shown substantially improved survival with both selective ET-A and nonselective ET-A/B receptor antagonists. In rats with CHF induced by coronary artery ligation, though acute ET-A receptor blockade with BQ 123 reduced myocardial contractility,[59] long term treatment resulted in a 90% survival rate, compared with 40% in nontreated animals.[58] This improved survival was associated with an amelioration of LV dysfunction and prevention of ventricular remodelling. Treatment with the nonselective receptor antagonist bosentan for 9 months in a coronary artery ligation model of CHF resulted in beneficial alterations in haemodynamics, cardiac geometry and function and an 18% increase in survival compared with controls.[68] In the Dahl salt-sensitive, hypertensive model of CHF, long term treatment with bosentan, started at the stage of compensated LVH, did not alter LV mass but improved survival by 36%.[62] Of note, in a pacing CHF model, long term (6 weeks) nonselective receptor antagonism (L 753037) increased coronary sinus NO concentrations and improved endothelium-dependent relaxation in coronary arteries.[69]

In humans, the increase in circulating big ET-1 or ET-1 seen in patients with CHF is correlated with the severity of symptoms and with prognosis.[70,71]

A series of clinical trials point towards potential usefulness of ET receptor antagonists in this condition (table II), though it is unclear whether selective or nonselective receptor antagonism will provide the greatest benefit.

Studies with bosentan have consistently reported marked haemodynamic benefits both acutely and in the longer term.[72-74] In patients on chronic triple therapy of ACE inhibitors, diuretic and digoxin, acute administration of intravenous bosentan (300mg in total) 2 days after withdrawal of the ACE inhibitor, resulted in a 33% decrease in pulmonary vascular resistance (PVR).[72] Oral bosentan (1g twice daily for 2 weeks) improved systemic vascular resistance index (SVR; –25%), PVR (–20%) and cardiac index (CI; +15%) on day 1 of treatment. After 2 weeks of continuous oral therapy, additional haemodynamic improvements (PVR; –10%, SVR; –10%, CI; +15%) were documented in patients receiving ongoing ACE inhibitor therapy.[73] The REACH study, in which 370 patients with CHF received bosentan (500mg twice daily) or placebo for 6 months, was terminated early because of asymptomatic, reversible increases in hepatic transaminases in the treatment group, but improvements in symptoms, and fewer hospitalisations were reported in bosentan-treated patients who completed the 6 month protocol.[74] Further clinical studies of bosentan in CHF are being planned.

The theoretical benefits of selective ET-A receptor antagonism in patients with CHF have, so far, only been explored in small acute studies. Intravenous BQ 123 (100 nmol/min for 60 min for 2 patients; 200 nmol/min for 60 min for 8 patients)[75] and TBC 11251 (1.5 or 3 mg/kg)[77] have produced significant systemic and pulmonary haemodynamic benefits, respectively (table II). Acute ET-B receptor antagonism with BQ 788, caused systemic vasoconstriction in CHF patients, an effect which was reversed by the addition of BQ 123, suggesting that ET-B receptors contribute to vasodilator tone in CHF.[76] Longer term studies with selective ET-A receptor antagonists have yet to be performed. Similarly, the long term effects of ET antagonism on vascular and ventricular remodelling remain to be explored in patients with CHF.

5. Hypertension

Exogenous administration of ET-1 increases peripheral vascular resistance and elevates blood pressure, and studies with ET receptor antagonists have revealed that endogenous ET-1 has an important role in the maintenance of normal vascular tone. It is also possible that ET-1 may modulate blood pressure by renal mechanisms, by altering the balance between its potentially favourable natriuretic action (ET-B receptors)[78] and sodium retention as a consequence of renal vasoconstriction (ET-A

Table II. Studies with endothelin receptor antagonists in patients with congestive heart failure (CHF)

Trial	Study design	n	Drug	Route and duration of administration	NYHA	MAP	PAP	SVR	PVR	HR	CI
Kiowski et al.[72]	nb, nc	24	Bosentan	IV bolus	III	↓	↓	↓	↓	↔	↑
Sütsch et al.[73]	r, db, pc	36	Bosentan	Oral 14 days	III	↓	↓	↓	↓	↔	↑
Packer et al.[74]	r, db, pc	370	Bosentan	Oral 6 months	IIIB-IV	Clinical improvement = reduced hospital admissions					
Cowburn et al.[75]	nb, nc	10	BQ 123	IV bolus	Stable CHF	↓	↓	↓	(↓)	↔	↑
Cowburn et al.[76]		8	BQ 788	IV bolus	Stable CHF	↑	(↑)	↑	(↑)	ns	↓
		7[a]	BQ 788 + BQ 123	IV bolus		↓	↓	↓	↓	ns	↑
Givertz et al.[77]	r, db, pc	24	TBC 11251	IV bolus	III-IV	↔	↓	↔	↓	↔	ns

a Seven patients of the original 8 received BQ 123 + BQ 188.

CI = cardiac index; **db** = double-blind; **HR** = heart rate; **IV** = intravenous; **MAP** = mean arterial pressure; **n** = number of participants; **nb** = nonblind; **nc** = noncomparative; **ns** = not specified; **NYHA** = New York Heart Association grade of CHF; **PAP** = pulmonary artery pressure; **pc** = placebo-controlled; **PVR** = pulmonary vascular resistance; **r** = randomised; **SVR** = systemic vascular resistance; ↑ = significant increase; ↓ = significant decrease; ↔ = no change; **(↑)** = nonsignificant increase; **(↓)** = nonsignificant decrease.

receptors),[26] by interactions with other hormone systems, such as the renin-angiotensin system,[79] by effects on the central and peripheral nervous system,[80] and, in the longer term, by its mitogenic properties, which will influence the calibre and responsiveness of blood vessels.[44,45] Knockout rodent models suggest that ET-B receptors participate in blood pressure regulation by causing vasodilatation and natriuresis.[15,78] Forearm models in healthy human volunteers have shown that selective ET-A receptor antagonism (BQ 123) causes local vasodilatation, combined ET-A and ET-B (BQ 123 plus BQ 788) or nonselective ET-A/B receptor antagonism (TAK 044) causes vasodilatation, but to a lesser extent than ET-A antagonism alone, whereas selective ET-B blockade (BQ 788) causes vasoconstriction.[81-84] Systemic studies with nonselective and selective ET-A or -B receptor antagonists have confirmed these local findings; selective ET-A (BQ 123) and nonselective receptor antagonists (TAK 044) produce similar reductions in mean arterial pressure and SVR while ET-B–selective receptor antagonists (BQ 788) produce an increase in peripheral vascular resistance.[85-87]

With respect to the pathophysiology of hypertension, production of ET-1 is increased in some (e.g. Dahl salt-sensitive and stroke-prone spontaneously hypertensive rats), but not all rat models.[88] Those models in which ET-1 production is increased (mostly, but not exclusively salt-dependent types) are associated with increased vascular growth and are responsive to both selective and nonselective ET receptor antagonists. This response comprises not only a modest reduction in blood pressure but also a marked regression of vascular growth.[88]

In humans, elevated plasma ET concentrations are found in hypertension, though this is not consistent.[89] These high concentrations would appear, mostly, to be a feature of severe hypertension or indicative of the presence of complications or coexisting disease. Levels of prepro-ET-1 and ECE mRNA are increased in the vascular smooth muscle cells of patients with hypertension,[6] and there is evidence that endothelial function, specifically endothelium-dependent vascular relaxation, is impaired both in patients with hypertension and in groups at risk of developing hypertension.[90,91] Forearm studies with selective ET-A or -B receptor antagonism (BQ 123 and BQ 788, respectively) suggest increased vascular ET activity in patients with essential hypertension compared with normotensive controls, and demonstrate a greater response to nonselective antagonism compared with selective ET-A antagonism.[92] These data need to be confirmed, but suggest a mechanism for ET in the pathogenesis of hypertension.

To date, one major study has examined the antihypertensive effects of an ET receptor antagonist in humans. In 293 patients with hypertension, oral bosentan 500 to 2000 mg/day for 4 weeks achieved a reduction in blood pressure equivalent to that achieved with 20mg of enalapril.[93] This reduction was achieved without a reflex increase in the activity of the sympathetic nervous system (as measured by plasma noradrenaline levels and heart rate) or renin-angiotensin system. Further confirmatory work is needed, but given the range of available antihypertensive drugs, a new class of agents would have to demonstrate greater efficacy, a better adverse effect profile, or other benefits in addition to blood pressure lowering properties in order to obtain a place in the therapy for hypertension. In addition to antihypertensive effects, the potential benefits of ET antagonists in patients with hypertension might include reducing long term structural changes in blood vessels (vascular hypertrophy or atherogenesis) or the myocardium, ameliorating endothelial dysfunction, and/or beneficial effects on salt and water balance. Subgroups such as salt-sensitive individuals or black patients with hypertension (with higher plasma ET levels and/or impaired endothelium-dependent relaxation[89,91]) might benefit the most from these agents.

6. Vasospastic Conditions

A role for ET has been suggested in vasospastic conditions, such as migraine and Raynaud's disease, because higher plasma ET concentrations are seen during active episodes in patients with these conditions.[80] It must be remembered, though, that ET

plays an important role in normal developmental biology.[78] ET antagonists are teratogenic in the first trimester of pregnancy and therefore use of these drugs in women of child-bearing age is precluded, limiting their usefulness in these and other conditions. However, this limitation is probably less important in the delayed cerebral vasospasm seen after subarachnoid haemorrhage or in ischaemic stroke. Selective and nonselective ET receptor antagonists (RO 611790 and SB 217242, respectively) have both been shown to reduce ischaemic brain injury in animals.[94-96] Phase II trials with ET antagonists for these indications are currently in progress.

7. Pulmonary Hypertension

ET-1 is elevated in animal models of pulmonary hypertension[97] and in humans, possibly because of increased pulmonary production, as the pulmonary venous concentration of ET-1 exceeds the arterial concentration.[98] In addition, tissue levels of mRNA for ET-1 appear to be correlated with the PVR.[99] Thus, pulmonary hypertension, a disease characterised by endothelial injury, vascular smooth muscle cell proliferation and pulmonary vasoconstriction, is another potential therapeutic target for ET receptor antagonists.

Nonselective and selective ET-A receptor antagonism both prevent N^G-monomethyl-L-arginine–induced pulmonary vasoconstriction in a rat monocrotaline model of pulmonary hypertension.[97] The lack of effect of ET-B antagonism suggests this is an ET-A-mediated effect. In a pig hypoxic model of pulmonary hypertension, selective ET-A and nonselective receptor antagonists were equally effective in countering hypoxia-induced increases in pulmonary artery pressure; ET-B-selective drugs had no effect.[100] Both ET-A–selective and nonselective drugs (table I) are currently in development for this indication.

8. Conclusion

ET receptor antagonists have been, and continue to be, crucial experimental tools in elucidating the role of the ET system in cardiovascular physiology and pathophysiology. With the increasing availability of orally administered selective and nonselective antagonists, it should be possible to more fully establish the clinical role of these drugs in patients with cardiovascular disease. If they live up to their promise, ET receptor antagonists could offer a wide range of benefits including the reduction of pre- and after-load, reduction of pulmonary and systemic blood pressure, improvement in coronary blood flow, and, in the longer term, a reduction in abnormal ventricular and vascular remodelling and atherosclerosis. Through these effects, they might be useful in the management of patients with CAD, hypertension and CHF. Though the cardiovascular system appears to be a promising target for these new drugs, further work is needed to clarify when selective as opposed to nonselective antagonism is required.

Acknowledgements

Dr J. Goddard is supported by an educational grant from Pfizer UK. Professor D.J. Webb is currently the recipient of a Research Leave Fellowship from the Wellcome Trust (WT 0526330).

References

1. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332 (6163): 411-5
2. Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A 1989; 86 (8): 2863-7
3. Arinami T, Ishikawa M, Inoue A, et al. Chromosomal assignments of the human endothelin family genes: The endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. Am J Hum Genet 1991; 48 (5): 990-6
4. Eid H, de Bold ML, Chen JH, et al. Epicardial mesothelial cells synthesize and release endothelin. J Cardiovasc Pharmacol 1994; 24 (5): 715-20
5. Kohan DE. Endothelins in the kidney: physiology and pathophysiology. Am J Kidney Dis 1993; 22 (4): 493-510
6. Rossi GP, Colona S, Pavan E, et al. Endothelin-1 and its mRNA in the wall layers of human arteries ex vivo. Circulation 1999; 99 (9): 1147-55
7. Brown MA, Smith PL. Endothelin: a potent stimulator of intestinal ion secretion *in vitro*. Regul Pept 1991; 36 (1): 1-19
8. Garvin J, Sanders K. Endothelin inhibits fluid and bicarbonate transport in part by reducing Na+/K+ ATPase activity in the rat proximal straight tubule. J Am Soc Nephrol 1991; 2 (5): 976-82

9. Haynes WG, Webb DJ. Endothelin as a regulator of cardiovascular function in health and disease. J Hypertens 1998; 16 (8): 1081-98
10. Arai H, Hori S, Aramori I, et al. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 1990; 348 (6303): 730-2
11. Sakurai T, Yanagisawa M, Takuwa Y, et al. Cloning of a cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. Nature 1990; 348 (6303): 732-5
12. Ogawa Y, Nakao K, Arai H, et al. Molecular cloning of a non-isopeptide-selective human endothelin receptor. Biochem Biophys Res Commun 1991; 178 (1): 248-255
13. Karet F, Kuc R, Davenport A. Novel ligands BQ 123 and BQ 3020 characterise endothelin receptor subtypes ETA and ETB in human kidney. Kidney Int 1993; 44 (1): 36-42
14. Gray GA, Webb DJ. The endothelin system and its potential as a therapeutic target in cardiovascular disease. Pharmacol Ther 1996; 72 (2): 109-48
15. Douglas SA. Clinical development of endothelin receptor antagonists. Trends Pharmacol Sci 1997; 18 (11): 408-12
16. Fukami T, Niiyami K, Amano Y, et al. Cyclic pentapeptide endothelin A receptor antagonists with attenuated *in vivo* clearance. Chem Pharm Bull 1996; 44 (3): 609-14
17. Webb ML, Meek TD. Inhibitors of endothelin. Med Res Rev 1997; 17 (1): 17-67
18. Liu G, Henry Jr KJ, Szczepankiewicz BG, et al. Pyrrolidine-3-carboxylic acids as endothelin antagonists: 3. Discovery of a potent, 2-nonaryl, highly selective ETA antagonists (A-216546). J Med Chem 1998; 41 (17): 3261-75
19. Wu C, Chan MF, Stavros F, et al. Discovery of TBC-11251, a potent, long-acting, orally active endothelin receptor-A selective antagonist. J Med Chem 1997; 40 (11): 1690-7
20. Hoshino T, Yamauchi R, Kikkawa K, et al. Pharmacological profile of T-0201, a highly potent and orally active endothelin receptor antagonist. J Pharmacol Exp Ther 1998; 286 (2): 643-9
21. Sorensen SS, Madsen JK, Pedersen EB. Systemic and renal effects of intravenous infusion of endothelin-1 in healthy human volunteers. Am J Physiol 1994; 266 (3 Pt 2): F411-8
22. Cirino M, Martin E, Yano M, et al. Cardiovascular and renal actions of the endothelin (B) receptor in pigs. J Cardiovasc Pharmacol 1997; 29 (6): 704-12
23. Moravec CS, Reynolds EE, Stewart RW, et al. Endothelin is a positive inotropic agent in human and rat heart *in vitro*. Biochem Biophys Res Commun 1989; 159 (1): 14-8
24. Pieske B, Beyermann B, Breu V, et al. Functional effects of endothelin regulation of endothelin receptors in isolated human nonfailing and failing myocardium. Circulation 1999; 99 (14): 1082-9
25. Beyer ME, Slesak G, Hovelborn T, et al. Inotropic effects of endothelin-1. Interaction with molsidomine and with BQ 610. Hypertension 1999; 33 (1): 145-52
26. Rabelink TJ, Kaasjager KA, Boer P, et al. Effects of endothelin-1 on renal function in humans: Implications for physiology and pathophysiology. Kidney Int 1994; 46 (2): 376-81
27. Kruger D, Sheikzadeh A, Giannitsis E, et al. Cardiac release and kinetics of endothelin after severe short-lasting myocardial ischaemia. J Am Coll Cardiol 1997; 30 (4): 942-6
28. Yasuda M, Kohno M, Tahara A, et al. Circulating immunoreactive endothelin in ischaemic heart disease. Am Heart J 1990; 119 (4): 801-6
29. Toyo-oka T, Aizawa T, Suzuki N, et al. Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. Circulation 1991; 83 (2): 476-83
30. Ray SG, McMurray JJ, Morton JJ, et al. Circulating endothelin in acute ischaemic syndromes. Br Heart J 1992; 67 (5): 383-6
31. Wieczorek I, Haynes WG, Webb DJ, et al. Raised plasma endothelin in unstable angina and non-Q wave myocardial infarction: relation to cardiovascular outcome. Br Heart J 1994; 72 (5): 436-41
32. Omland T, Lie RT, Aakvaag A, et al. Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction. Circulation 1994; 89 (4): 1573-9
33. Richards AM, Webb DJ, Yandle TG, et al. Comparison of amino terminal pro-brain natriuretic peptide and big endothelin-1 as prognostic indicators after myocardial infarction [abstract]. J Am Coll Cardiol 1999; 33 (2 Suppl. A): 194A
34. Seo B, Oemar BS, Siebenmann R, et al. Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. Circulation 1994; 89 (3): 1203-8
35. Awane-Igata Y, Ikeda S, Watanabe T. Inhibitory effects of TAK-044 on endothelin induced vasoconstriction in various canine arteries and porcine coronary arteries: a comparison with selective ETA and ETB receptor antagonists. Br J Pharmacol 1997; 120 (3): 516-22
36. Wenzel RR, Fleisch M, Shaw S, et al. Hemodynamic and coronary effects of the endothelin antagonist bosentan in patients with coronary artery disease. Circulation 1998; 98 (21): 2235-40
37. Grover GJ, Dzonczyk S, Parham CS. The endothelin-1 receptor antagonist BQ-123 reduces infarct size in a canine model of coronary occlusion and reperfusion. Cardiovasc Res 1993; 27 (9): 1613-8
38. Watanabe T, Awane Y, Ikeda S, et al. Pharmacology of a non-selective ETA and ETB receptor antagonist, TAK 044 and the inhibition of myocardial infarct size in rats. Br J Pharmacol 1995; 114 (5): 949-54
39. Lerman A, Edwards BS, Hallett JW, et al. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. N Engl J Med 1991; 325 (14): 997-1001
40. Zeiher AM, Gobel H, Schachinger V, et al. Tissue endothelin-1 immunoreactivity in the active coronary atherosclerotic plaque. A clue to the mechanism of increased vasoreactivity of the culprit lesion in unstable angina. Circulation 1995; 91 (4): 941-7
41. Kowala MC, Rose PM, Stein PD, et al. Selective blockade of the endothelin subtype A receptor decreases early atherosclerosis in hamsters fed cholesterol. Am J Pathol 1995; 146 (4): 819-26
42. Barton M, Haudenschild CC, D'Uscio LV, et al. Endothelin ET(A) receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. Proc Natl Acad Sci USA 1998; 95(24): 14367-72
43. Best PJM, McKenna CJ, Hasdai D, et al. Chronic endothelin receptor antagonism preserves coronary endothelial function in experimental hypercholesterolaemia. Circulation 1999; 99 (13): 1747-52
44. Hirata Y, Takagi Y, Fukuda Y, et al. Endothelin is a potent mitogen for rat vascular smooth muscle cells. Atherosclerosis 1989; 78 (2-3): 225-8
45. Weissberg PL, Witchell C, Davenport AP, et al. The endothelin peptides ET-1, ET-2, ET-3 and sarafotoxin S6b are co-mitogenic with platelet-derived growth factor for vascular smooth muscle cells. Atherosclerosis 1990; 85 (2-3): 257-62
46. Wang X, Douglas SA, Louden C, et al. Expression of endothelin-1, endothelin-3, endothelin-converting enzyme-1, and endothelin-A and endothelin-B receptor mRNAs after angioplasty induced neointimal formation in the rat. Circ Res 1996; 78 (2): 322-8
47. Douglas SA, Louden C, Vickery-Clark LM, et al. A role for endogenous endothelin-1 in neointimal formation after rat

carotid artery balloon angioplasty. Protective effects of the novel non-peptide endothelin receptor antagonist SB 209670. Circ Res 1994; 75 (1): 190-7
48. Tahara A, Kohno M, Yanagai S, et al. Circulating immunoreactive endothelin in patients undergoing percutaneous transluminal coronary angioplasty. Metabolism 1991; 40 (12): 1235-7
49. Hasdai D, Holmes DRJ, Garratt KN, et al. Mechanical pressure and stretch release endothelin-1 from human atherosclerotic coronary arteries *in vivo*. Circulation 1997; 95 (2): 357-62
50. Douglas SA, Vickery-Clarke LM, Louden C, et al. Endothelin receptor subtypes in the pathogenesis of angioplasty-induced neointima formation in the rat: a comparison of selective ETA receptor antagonism and dual ETA/ETB receptor antagonism using BQ-123 and SB 209670. J Cardiovasc Pharmacol 1995; 26 (3 Suppl.): S186-9
51. Chandra S, Sarkar S, Elliott JD, et al. Application of magnetic resonance imaging for evaluation of the efficacy of SB 217242 in neointimal formation. J Cardiovasc Pharmacol 1998; 31 (1 Suppl.): S317-9
52. McKenna CJ, Burke SE, Opgenorth TJ, et al. Selective ET(A) receptor antagonism reduces neointimal hyperplasia in a porcine coronary stent model. Circulation 1998; 97 (25): 2551-6
53. Douglas SA, Nichols AJ, Feuerstein GZ, et al. SB 209670 inhibits the arrhythmogenic actions of endothelin-1 in the anesthetized dog. J Cardiovasc Pharmacol 1998; 31 (1 Suppl.): S99-102
54. Raschack M, Juchelka F, Rozek-Schaefer G. The endothelin-A antagonist LU 135 252 suppresses ischaemic extrasystoles and fibrillation in pigs and prevents hypoxic cellular decoupling. J Cardiovasc Pharmacol 1998; 31 (1 Suppl.): S145-8
55. Garjani A, Wainwright CL, Zeitlin I, et al. Effects of endothelin-1 and the ET(A) receptor antagonist BQ-123, on ischaemic arrhythmias in anesthetized rats. J Cardiovasc Pharmacol 1995; 25 (4): 634-42
56. Kramer BK, Ittner ME, Beyer ME, et al. Circulatory and myocardial effects of endothelin. J Mol Med 1997; 75 (11-12): 886-90
57. Prasad MR. Endothelin stimulates degradation of phospholipids in isolated rat hearts. Biochem Biophys Res Commun 1991; 174 (2): 952-7
58. Sakai S, Miyauchi T, Kobayashi M, et al. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. Nature 1996; 384 (6607): 353-5
59. Sakai S, Miyauchi T, Sakurai T, et al. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats with congestive heart failure. Marked increase in endothelin-1 production in the failing heart. Circulation 1996; 93 (6): 1214-22
60. Wei CM, Clavell AL, Burnett JCJ. Atrial and pulmonary endothelin mRNA is increased in a canine model of chronic low cardiac output. Am J Physiol 1997; 273 (2-2): R838-44
61. Tonnessen T, Lunde PK, Giaid A, et al. Pulmonary and cardiac expression of preproendothelin-1 mRNA are increased in heart failure after myocardial infarction in rats. Localization of preproendothelin-1 mRNA and endothelin peptide. Cardiovasc Res 1998; 39 (3): 633-43
62. Iwanaga Y, Kihara Y, Hasegawa K, et al. Cardiac endothelin-1 plays a critical role in the functional deterioration of left ventricles during the transition from compensatory hypertrophy to congestive heart failure in salt-sensitive hypertensive rats. Circulation 1998; 98 (19): 2065-73
63. Teerlink JR, Breu V, Sprecher U, et al. Potent vasoconstriction mediated by endothelin ETB receptors in canine coronary arteries. Circ Res 1994; 74 (1): 105-14
64. Cannan CR, Burnett Jr JC, Lerman A. Enhanced coronary vasoconstriction to endothelin-B-receptor activation in experimental congestive heart failure. Circulation 1996; 93 (4): 646-51
65. Love MP, Haynes WG, Gray GA, et al. Vasodilator effects of endothelin-converting enzyme inhibition and endothelin ETA receptor blockade in chronic heart failure patients treated with ACE inhibitors. Circulation 1996; 94 (9): 2131-7
66. Kelso EJ, Geraghty RF, McDermott BJ, et al. Mechanical effects of ET-1 in cardiomyocytes isolated from normal and heart-failed rabbits. Mol Cell Biochem 1996; 157 (1-2): 149-55
67. Thomas PB, Liu EC, Webb ML, et al. Exogenous effects and endogenous production of endothelin in cardiac myocytes: potential significance in heart failure. Am J Physiol 1996; 271 (6 Pt 2): H2629-37
68. Mulder P, Richard V, Derumeaux G, et al. Role of endogenous endothelin in chronic heart failure: effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodelling. Circulation 1997; 96 (6): 1976-82
69. Shen Y-T, Buie P, Moussa T, et al. Chronic therapy with L-753,037, an ETA/B receptor antagonist, in conscious dogs during development of heart failure [abstract]. Circulation 1998; 98 (17 Suppl. I): I-578
70. Pacher R, Bergler-Klein J, Globits S, et al. Plasma big endothelin-1 concentrations in congestive heart failure patients with or without systemic hypertension. Am J Cardiol 1993; 71(15): 1293-9
71. Wei CM, Lerman A, Rodeheffer RJ, et al. Endothelin in human congestive heart failure. Circulation 1994; 89 (4): 1580-6
72. Kiowski W, Sütsch G, Hunziker P, et al. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. Lancet 1995; 346 (8977): 732-6
73. Sütsch G, Kiowski W, Yan X-W, et al. Short-term oral endothelin-receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. Circulation 1998; 98 (1): 2262-8
74. Packer M, Caso A, Charlton V, et al. Multicenter, double-blind, placebo-controlled study of long-term endothelin blockade with Bosentan in chronic heart failure – results of the REACH-1 trial [abstract]. Circulation 1998; 98 (17 Suppl. I): I-3
75. Cowburn PJ, Cleland JGF, McArthur JD, et al. Short-term haemodynamic effects of BQ-123, a selective endothelin ETA-receptor antagonist, in chronic heart failure. Lancet 1998; 352 (9123): 201-2
76. Cowburn PJ, Cleland JGF, McDonagh TA, et al. Adverse hemodynamic effects of a selective endothelin ETB receptor antagonist in patients with chronic heart failure: reversal with a selective endothelin ETA receptor antagonist. Circulation 1998; 98 (17 Suppl. I): I-718
77. Givertz MM, Colucci WS, Gottlieb SS, et al. Acute ETA receptor blockade reduces pulmonary vascular resistance in patients with chronic heart failure [abstract]. Circulation 1998; 98 (17 Suppl. I): I-578
78. Webb DJ, Monge JC, Rabelink TJ, et al. Endothelin: new discoveries and rapid progress in the clinic. Trends Pharmacol Sci 1998; 19 (1): 5-8
79. Rakugi H, Tabuchi Y, Nakamaru M, et al. Endothelin activates the vascular renin-angiotensin system in rat mesenteric arteries. Biochem Int 1990; 21 (5): 867-72
80. Ferro CJ, Webb DJ. The clinical potential of endothelin receptor antagonists in cardiovascular medicine. Drugs 1996; 51 (1): 12-27

81. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone. Lancet 1994; 344 (8926): 852-4
82. Haynes WG, Strachan FE, Webb DJ. Endothelin ETA and ETB receptors cause vasoconstriction of human resistance and capacitance vessels *in vivo*. Circulation 1995; 92 (3): 357-63
83. Haynes WG, Strachan FE, Gray GA, et al. Forearm vasoconstriction to endothelin-1 is mediated by ETA and ETB receptors *in vivo* in humans. J Cardiovasc Pharmacol 1995; 26 (3 Suppl.): S40-3
84. Verhaar MC, Strachan FE, Newby DE, et al. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. Circulation 1998; 97 (8): 752-6
85. Haynes WG, Ferro CJ, O'Kane KPJ, et al. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. Circulation 1996; 93 (10): 1860-70
86. Strachan FE, Spratt JC, Wilkinson IB, et al. Systemic blockade of the endothelin-B receptor increases peripheral resistance in healthy men. Hypertension 1999; 33 (1 Pt 2): 581-5
87. Spratt JC, Goddard J, Labinjoh C, et al. Systemic blockade of the ETA receptor decreases peripheral vascular resistance in healthy men [abstract]. Br J Clin Pharmacol 1999; 47: 575P
88. Schiffrin EL. Endothelin and endothelin antagonists in hypertension. J Hypertens 1998; 16 (12II): 1891-5
89. Goddard J, Webb DJ. Plasma endothelin concentrations in hypertension. J Cardiovasc Pharmacol 1999. In press
90. Cardillo C, Kilcoyne CM, Quyyumi AA, et al. Selective defect in nitric oxide synthesis may explain the impaired endothelium-dependent vasodilation in patients with essential hypertension. Circulation 1998; 97 (9): 851-6
91. Cardillo C, Kilcoyne CM, Cannon RO, et al. Attenuation of cyclic nucleotide-mediated smooth muscle relaxation in blacks as a cause of racial differences in vasodilator function. Circulation 1999; 99 (1): 90-5
92. Cardillo C, Kilcoyne CM, Waclawiw M, et al. Role of endothelin in the increased vascular tone of patients with essential hypertension. Hypertension 1999; 33 (2): 753-8
93. Krum H, Viskoper RJ, Lacourciere Y, et al. The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. N Engl J Med 1998; 338 (12): 784-90
94. Barone FC, White RF, Elliot JD, et al. The endothelin receptor antagonist SB 217242 reduces cerebral focal ischaemic brain injury. J Cardiovasc Pharmacol 1995; 26 (3 Suppl.): S404-7
95. Roux S, Breu V, Giller T, et al. Ro 61-1790, a new hydrosoluble endothelin antagonist: general pharmacology and effects on experimental cerebral vasospasm. J Pharmacol Exp Ther 1997; 283 (3): 1110-8
96. Dawson DA, Hallenbeck JM, Spatz M. The endothelin antagonist Ro 61-1790 attenuates focal cerebral ischaemic injury. Stroke 1998; 29: 323
97. Frasch HF, Marshall C, Marshall BE. Endothelin-1 is elevated in monocrotaline pulmonary hypertension. Am J Physiol 1999; 276 (2 Pt 1): L304-10
98. Stewart DJ, Levy RD, Cernacek P, et al. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? Ann Int Med 1991; 114: 464-9
99. Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in lungs of patients with pulmonary hypertension. N Engl J Med 1993; 328 (24) : 1732-9
100. Franco-Cereceda A, Holm P. Selective or nonselective endothelin antagonists in porcine hypoxic pulmonary hypertension. J Cardiovasc Pharmacol 1998; 31 Suppl. 1: S447-52

Correspondence and reprints: Professor *David J. Webb*, Clinical Pharmacology Unit & Research Centre, Department of Medical Sciences, The University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland.
E-mail: d.j.webb@ed.ac.uk

Journal of Cardiovascular Pharmacology™
35 (Suppl. 2):S25–S31 © 2000 Lippincott Williams & Wilkins, London

Plasma Endothelin Concentrations in Hypertension

Jane Goddard and David J. Webb

Clinical Pharmacology Unit & Research Centre, Department of Medical Sciences, University of Edinburgh, Edinburgh, Scotland, U.K.

Summary: The endothelins comprise a family of potent vasoconstricting peptides. Endothelin-1 appears to be the predominant isoform produced by the vascular endothelium, acting mainly in a paracrine fashion on vascular smooth muscle cells to cause vasoconstriction. It also has a range of other local actions – in the kidney, in the nervous system and on other hormone systems – that could, potentially, play a part in the genesis of hypertension. The association of raised plasma endothelin concentrations in human hypertension has caused much interest, but the literature is not consistent. Given the generally low plasma concentration of the endothelins, and their mainly paracrine actions, it remains unclear whether plasma endothelin has a functional role in hypertension. Additionally, problems remain with the measurement of plasma endothelin that raise doubts about the validity of conclusions drawn from these measurements.
Key Words: Plasma—Endothelin—Hypertension—Radioimmunoassay—Enzyme-linked immunoassay.

INTRODUCTION

The endothelins (ETs) are a family of 21-amino-acid peptides with vasoactive, inotropic and mitogenic properties and the ability to modulate other hormone systems, as well as gut, kidney and lung function. Three different peptides, endothelin (ET)-1, ET-2 and ET-3, each with distinct genes and tissue distributions, have so far been identified. ET-2 differs from ET-1 by two amino acids and ET-3 from ET-1 by six amino acids. Each has a common structure of a hydrophobic C terminal end and two intrachain disulphide bonds that form a hairpin structure (1–4).

ET-1 is primarily produced by endothelial cells but is also found in a variety of other cell types in heart (epicardium), lung (bronchial epithelial cells), kidney (tubular cells), central and peripheral nervous system tissue (astrocytes/nerve plexi/pituitary tissue), macrophages and vascular smooth muscle cells. ET-2 mRNA is found to a lesser extent in endothelial cells and also in other cells in heart and kidney. ET-3 is found in gut, kidney, adrenal secretory tissue, macrophages and CNS tissue but not endothelium (2,5–7).

ET-1 is synthesized from preproendothelin-1, the 212-amino-acid gene product. Endothelin genes are thought to be regulated at the transcription level with stimuli to gene transcription including hormones (such as adrenaline, angiotensin II, insulin and arginine vasopressin), cytokines (such as platelet-derived growth factor and transforming growth factor beta), hypoxia, oxidized low density lipoprotein and cyclosporin. Inhibition has been documented in response to prostacyclin, nitric oxide, atrial and other natriuretic peptides and heparin (4,7,8).

Preproendothelin-1 is processed to form proendothelin-1 which is then cleaved by intracellular furin-like proteases to generate big ET-1 (38 amino acids) which is biologically inactive. Endothelin-converting enzyme (ECE), a metalloprotease, then cleaves big ET-1 to the biologically active ET-1 and a 17-amino-acid C-terminal fragment-1 (CTF-1). A group of ECEs for ET-1 have been identified, each with a different tissue distribution and different pH at which they act. ECEs have not yet been identified that are selective for ET-2 and ET-3 (9).

Two receptors for ET have been identified. ET_A receptors are found in the aorta, heart, kidney and vascular smooth muscle cells where their activation causes cell contraction. They exhibit a marked selectivity for ET-1. ET_B receptors are present on endothelial cells where their activation results in the production of nitric oxide and prostacyclin, resulting in vasodilatation. They are also present on vascular smooth muscle cells where their acti-

Address correspondence and reprint requests to Professor David Webb at the Clinical Pharmacology Unit & Research Centre, Department of Medicine, The University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland, U.K.

vation causes vasoconstriction. They have equal affinity for ET-1, ET-2 and ET-3. Signal transduction is via G protein coupling and activation of intracellular phospholipase-C with subsequent hydrolysis of phosphatidyl inositol and changes in intracellular calcium. Removal of ET-1 from the circulation is by receptor binding and internalization (ET_B effect), renal clearance and enzymatic degradation by neutral endopeptidases (8–10).

MEASUREMENT

Plasma endothelin-like immunoreactivity is said to comprise around 60% big ET-1 and 30% ET-1. ET-3 but not ET-2 also circulates in the blood but at lower concentrations (~10%) (4,11,12). Most of the focus has been on plasma ET-1 because it is the major active isoform in blood, although it is possible that big ET-1 could act as a circulating hormone which is processed to ET-1 at its site of action.

There are many problems associated with the measurement and interpretation of plasma ET-1 concentrations. Most ET-1 (~90%) is released from endothelial cells abluminally (13) where it acts on endothelial and smooth muscle cells as a predominantly autocrine and paracrine mediator. Plasma ET-1 may not, therefore, be an accurate reflection of endothelial synthesis of ET-1. In addition, the ET-1 concentration in plasma is likely to reflect a balance between production/overspill and its clearance. Under normal circumstances, ET-1 is rapidly cleared from the circulation by ET_B receptors, the kidney and neutral endopeptidases and so plasma concentrations may be largely determined by production. Consistent with this, although ET-1 exerts effects for up to 60 min, the plasma half-life in healthy subjects is only ~1 min (14). Reduction in ET_B receptor numbers, enzymatic degradation, or renal function, or administration of an ET_B receptor antagonist, may reduce this clearance and increase plasma ET-1 half-life.

Hence, in a number of situations, plasma ET-1 concentrations can be altered without a change in ET-1 production. It may not, therefore, be valid to draw conclusions about ET-1 production from plasma concentrations. Plasma big ET-1 has been proposed as a more reliable measure of endothelial ET-1 synthesis because its removal from the circulation is slower and less variable (15,16).

Turning to measurement techniques, plasma ETs are currently analysed by either radioimmunoassay (RIA) or enzyme-linked immunoassay (ELISA). Because normal plasma concentrations are in the low picomolar range (12,17–25), direct measurement in the plasma is difficult and both RIA and ELISA require ET to be extracted from the plasma and concentrated prior to measurement. Recovery rates during extraction are variable depending on, for example, the size and nature of the extraction columns and the fluid used to elute the ET; acetic acid extraction (26) achieves the highest recovery rate of 90% but recovery can be as low as around 50% (see Table 1). ET-1 is also partly degraded during the extraction process (26), further reducing the amount of plasma ET-1 that will then be assayed.

Antibodies commonly used in current assays exhibit cross-reactivity between the subtypes of ET, depending on which part of the molecule the antibody is raised against and depending on the antibody used; for example, using ET-1 detection of 100% as the standard, cross-reactivity of the antibody can range between 0 and 200% with big ET-1, between 10 and 1300% for ET-2, and between 0% and 120% for ET-3 (see Table 1). Many papers quote the concentration of 'plasma ET-1', when a more realistic term, given the cross-reactivity of the antibodies used, is 'plasma ET-like immunoreactivity'. As a separate issue, it is not clear whether the ET assayed in blood comprises mature peptides or degraded fragments of these. Therefore, it is possible that the high plasma ET-1 concentrations, for instance in renal failure, may bind ET antibodies but not be biologically active.

The sensitivity of a good immunoassay may be sufficient to detect levels down to 0.5 pg/ml. However, some kits have a sensitivity no lower than 8 pg/ml, higher than the range in which blood concentrations in healthy subjects are found. Also, intra- and inter-assay variability tends to be relatively high for ET assays, with inter-assay variations of > 10% reported, thus reducing the precision of the measurement (see Table 1). In addition, sample handling is important because haemolysis can increase measured ET concentrations tenfold. Lipaemic or icteric samples also give falsely high readings. Furthermore, dilution curves of synthetic pure ET-1 from different suppliers also show considerable variation (26). Hence, the reference point for biological samples varies depending on the supplier of the standard. Finally, the conditions under which blood is sampled must be borne in mind given that changes in position (45), temperature (24) and mental stress (46) have been reported to affect plasma ET-1 concentrations, and given the known interaction between ET-1 and other neurohumoral mechanisms (41,47,48). Taking these many factors into account, comparisons between studies performed in different laboratories are currently probably not warranted, although the ratios of concentrations in patients relative to controls may be a useful measure (Table 2).

PLASMA ENDOTHELIN AND ESSENTIAL HYPERTENSION

ET-1 is a profound vasoconstrictor, causes cell proliferation, and is involved in the maintenance of basal vascular tone in a balance between constricting and dilating substances. It also activates the sympathetic nervous system and the renin–angiotensin–aldosterone axis. Hence, a role has been sought for the peptide in the pathophysiology of essential hypertension, a condition characterized by endothelial dysfunction and increased vascular tone (1). In this review, we have considered measurements of plasma ET concentration in patients with essential hypertension in all papers published in English or abstracted in English that appear on *Medline*. Although plasma ET-1 levels have been reported as elevated in patients with essential hypertension in some

TABLE 1. Plasma endothelin concentrations in essential hypertension: assay employed

Study (ref.)	Difference between essential hypertension and control detected?	Number of patients/ controls	Recovery (during extraction process)	Assay	Inter-assay coefficient of variation	Intra-assay coefficient of variation	Cross-reactivity – as stated in papers ET-2	ET-3	Big ET
Kohno 1990 (11)	Yes (not borderline group) +ve correlation with BP	54/25	63%	Peninsula RIA	Not stated	Not stated	84%	5%	14%
Saito 1990 (27,28)	Yes (especially with target organ damage)	20/12	52–55%	Monoclonals RIA	6.8%	7.3%	80–100%	3-60%	50–100%
Shichiri 1990 (20)	Yes	11/21	61%	RIA	13%	10%	100%	100%	NS
Naruse 1991 (29)	Yes (in patients with target organ damage)	82/91	90%	Dainippon RIA	Not stated	Not stated	277%	< 0.1%	80%
Widimsky 1991 (30)	Yes (in severe hypertension; +ve correlation with BP)	16/8	Not stated	Amersham RIA	Not stated	Not stated	200%	< 0.002%	38%
Fernandez-Cruz 1993 (31)	Yes (in young but not old)	62/119	78%	Peninsula RIA	15%	12%	7%	7%	17%
Fogari 1994 (32)	+ve correlation with ABPM (no control group)	15	Not stated	Amersham RIA	9%	6%	204%	0.024%	38%
Lemne 1994 (33)	Yes (borderline hypertensives)	75/75	Not stated	RIA	8%	5%	32%	120%	6%
Januszewicz 1994 (34)	Yes (mild to moderate hypertensives)	37/21	100%	Amersham RIA	13.8%	4.8%	Not stated	Not stated	Not stated
Zocalli 1995 (25)	Yes (and +ve correlation with DBP)	20/8	75–85%	Peninsula RIA	15%	8%	7%	7%	35%
Ergul 1996 (35)	Yes (particularly black hypertensives)	50/50	Direct measurement	ELISA	3.5%	3.3%	100%	<5%	1%
Zaporowska-Stachowiak 1997 (36)	Yes	64/44	Not stated	Amersham RIA	Not stated	Not stated	1305%	0.39%	189%
Davenport 1990 (37)	No (-ve correlation with BP)	25/25	73%	Amersham RIA	13%	9%	200%	<0.002%	38%
Predel 1990 (19)	No (mild to moderate hypertensives)	12/20	'almost complete'	Peninsula RIA	Not stated	Not stated	'100% with endothelin'		
Schiffrin 1991 (38)	No (mild to severe hypertensives; +ve correlation with BP)	22/17	75%	Peninsula RIA	Not stated	Not stated	Not stated	7%	10%
Miyauchi 1992 (39)	No	51/75	Not stated	ELISA (Banyu)	Not stated	Not stated	Not stated	NS	NS
Baldys-Waligorska 1993 (40)	No (mild hypertensives; +ve correlation with BP)	10/36	88%	Biomedica RIA	8%	5%	100%	100%	0%
Veglio 1993 (22)	No (mild to moderate hypertensives)	25/30	90%	Peninsula RIA	9%	6%	7%	7%	17%
Hoffman 1994 (23)	No	17/19	85–92%	Peninsula RIA	18%	7%	7%	7%	35%
Haynes 1994 (41)	No	12/12	84%	RIA	4.2%	2.4%	52%	96%	7%
Sorensen 1994 (42)	No	12/12	85%	RIA	11%	8%	54%	54%	Not stated
Ferri 1995 (43)	No (obese)	15/10	85%	Peninsula RIA	10%	10%	7%	7%	17%
	No (non-obese)	12/11							
Letizia 1995 (24)	No (borderline hypertensives)	10/10	90%	Peninsula RIA	Not stated	Not stated	7%	7%	Not stated
Schiffrin 1997 (44)	No (trend to higher in severe hypertensives)	8/5	75%	Peninsula RIA	Not stated	Not stated	Not stated	7%	10%

NS, no significant cross-reactivity; BP, blood pressure; DBP, diastolic blood pressure, ABPM, ambulatory blood pressure monitoring; RIA, radioimmunoasssay; ELISA, enzyme-linked immunoassay.

TABLE 2. Plasma immunoreactive endothelin concentrations [adapted from Nelson et al. (49)]

Disease state	Reference	Number of subjects (controls)	Plasma ET (pg/ml)	Ratio (disease/normal)
Systemic sclerosis	Yamane 1992 (50)	31 (25)	1.9	1.5
Advanced atherosclerosis	Lerman 1991 (51)	40 (100)	8.0	2.3
Advanced prostate cancer	Nelson 1995 (49)	79 (26)	13.2	2.6
Sepsis	Pittet 1991 (52)	11 (14)	19.9	3.3
Congestive cardiac failure	Wei 1994 (53)	8 (6)	23.2	3.3
Acute myocardial infarction	Yasuda 1990 (54)	9 (25)	3.8	7.6
Hepatorenal syndrome	Moore 1992 (55)	11 (11)	36.5	9.1
Haemangioendothelioma	Yokokawa 1991 (56)	2 (8)	12.7	12.7
Cardiogenic shock	Cernacek 1989 (57)	6 (14)	3.7	14.0

studies (11,20,25,27–36,58), this is by no means universal (19,22–24,37–44).

Plasma ET concentrations, even when elevated, are generally not as high as those associated with haemodynamic effects when ET-1 is given exogenously in man (59–61). However, when ET-1 is given exogenously there is likely to be a gradient of concentration of ET-1 between blood and the endothelium/smooth muscle interface, such that the vascular smooth muscle is exposed to less ET-1 than is indicated by assays of plasma concentration. In contrast, the gradient is in the opposite direction for endogenously generated ET-1, so that the concentration in plasma is considerably less than at the endothelium/smooth muscle interface. Therefore, for the same plasma concentration, vascular smooth muscle 'sees' considerably more ET-1 when it is generated endogenously than given exogenously. Indeed, in prostatic carcinoma, where plasma ET-1 concentrations are as high as 50 pg/ml, but are probably not generated at the endothelium/smooth muscle interface, blood pressure is not raised (49). Hence, comparisons based on plasma concentrations are unlikely to be valid and locally increased ET-1 generation may occur in the blood vessels of hypertensive subjects without it being clearly reflected in the blood, and may have important effects on vascular structure and function, either directly or indirectly, such as through potentiation of the sympathetic nervous system (41).

Why do only some studies show an elevated plasma ET concentration in hypertension? It may relate to the use of different assays with different cross-reactivities. First, if the main plasma ET species are ET-1 and big ET-1, with a smaller contribution from ET-3, then those assays purporting to measure ET-1 but with a high cross-reactivity for these other species might give artificially high values. However, this does not appear to explain the disagreement because even those groups using antibodies with a low (< 10%) cross-reactivity for these species are divided in their findings with respect to plasma ET-1 concentrations in hypertension (see Table 1). Second, two groups have reported that the plasma ET immunoreactivity in patients with essential hypertension has the same high performance liquid chromatography (HPLC) profile as in normotensives i.e. the same relative concentrations of the ET subtypes (11,62). Hence, inaccuracy in ET-1 measurement resulting from cross-reactivity should be the same for hypertensive and normotensive groups and a true difference, therefore, still exist whatever the cross-reactivity of the antibody used in the assay.

Does the size of the populations studied account for the difference of opinion? Davenport et al. (37) studied 25 patients and 25 controls and showed no differences in plasma ET-1 concentrations. They calculated that for the power of the test to be able to detect a 25% difference in concentrations, at a 5% significance level, 100 patients would be required. No study meets this size requirement. From Table 1 it can be seen that, although there is one larger study failing to detect an elevation in plasma ET-1 in hypertensives, the majority of larger studies are in the group that do detect a difference. Whereas the published studies mostly account for other important confounding factors, including age, creatinine and antihypertensive drugs, a meta-analysis is likely to be unhelpful because the assays and the populations are not comparable, or sufficiently defined, and a strong possibility of publication bias for the positive studies exists.

It does appear, though, fairly consistently, that patents with severe hypertension are much more likely to have elevated plasma ET-1 concentrations. Several groups who report a difference in plasma (ET-1) note this is limited to patients with more advanced disease, in terms of target organ damage or higher blood pressures (11, 28–30,58). Additionally, one group who observed no significant difference did see a trend towards higher plasma ET-1 concentrations in severe disease (44). This could be explained by the observation that patients with atherosclerosis (a common condition in hypertensive patients) have higher circulating ET-1 concentrations irrespective of blood pressure (51) but groups specifically excluding patients with vascular disease have still detected this effect (25). If plasma ET-1 levels are higher in more severe hypertension, one might expect to find a correlation between plasma ET-1 and blood pressure. Some groups do observe such a correlation (11,25,30,31,33), even when no absolute difference has been detected (38,40) whereas some do not (19,23,42,43) even when a difference between groups is observed (28,29,34,35).

Indeed, one study observed a significant negative correlation between blood pressure and plasma ET-1 in the hypertensive group (37). Fogari et al. (32) did not find a correlation between plasma ET concentration and blood pressure measured in the clinic (three readings, supine) but did find a significant correlation when blood pressure was measured using 24-h ambulatory blood pressure monitoring. Interestingly, in relation to the plasma findings in severe hypertension, Schiffrin et al. (44) have recently demonstrated an increase in ET-1 gene expression in resistance arteries that is confined to patients with severe hypertension, and only associated with a trend for plasma ET-1 to be elevated, suggesting that ET-1 production may be increased in hypertension but this is poorly reflected by changes in plasma ET-1.

Ergul et al. (35) report a striking threefold higher plasma ET-1 concentration in black patients with hypertension compared to white patients with hypertension. Hoffman et al. (23) did not detect this racial difference but the numbers in each group in both studies were small and the issue needs clarification with a larger study. In salt-sensitive compared to salt-resistant patients with essential hypertension findings are similarly equivocal (23, 63). What is clear is that a substantial overlap in plasma ET-1 concentrations exists between the hypertensive group, however it is described, and the control group in all of the studies. Thus, with respect to plasma ET concentrations in essential hypertension, they are neither a sensitive nor a specific index of this condition.

Two groups have found abnormalities in ET-1 clearance in hypertensive patients with normal renal function, suggesting an abnormality in renal ET handling consistent with reduced ET-1 clearance or renal production (23,25). This is in marked contrast to the situation in renal failure where ET-1 clearance/glomerular filtration rate (GFR) ratio is substantially raised (25). Within the kidney, ET has the potential to generate hypertension by several mechanisms. The effect of exogenous ET-1 on the renal vasculature is to cause vasoconstriction (which will activate the renin–angiotensin system) and profound salt and water retention, both having the potential to raise blood pressure (64). It is not clear yet whether the elevated plasma ET-1 concentrations seen in various disease states are able to cause such renal vasoconstriction but it is known that ET-1 can cause a reduction in renal blood flow and GFR at concentrations that do not alter systemic haemodynamics (65). However, ET-1 is also produced by inner medullary collecting duct cells (IMCD) where it inhibits the arginine vasopressin (AVP) stimulated retention of water (66). There is evidence that extracellular sodium concentrations may regulate IMCD ET-1 production (67) and hence ET-1 may have a role in the renal regulation of volume homeostasis. If, as the urinary data suggest (23,25), nephron ET-1 production or handling is altered in essential hypertension, there may be inappropriate sodium and water retention aiding the development or maintenance of hypertension. Interestingly, spontaneously hypertensive rats have been shown to have reduced medullary ET-1 levels (68), whereas in the remnant kidney model of renal failure ET-1 production is increased (69).

CONCLUSIONS

Studies using receptor antagonists show that the ETs are important mediators in physiological blood pressure regulation in humans (70,71). Endothelin antagonists have also been shown to reduce blood pressure in hypertensive patients (72) and may have additional benefits in terms of potential effects on the heart, blood vessels and kidney (73). Measurement of plasma ET concentrations in hypertension is likely to be of limited value, and a poor reflection of ET-1 generation, although it may indicate hypertensive subgroups in whom ET antagonists might be particularly effective.

REFERENCES

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411–5.
2. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA* 1989;86:2863–7.
3. Arinami T, Ishikawa M, Inoue A, Yanagisawa M, Masaki T, Yoshida MC, Hamaguchi H. Chromosomal assignments of the human endothelin family genes: The endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene(EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. *Am J Hum Genet* 1991;48:990–6.
4. Kohno M, Yasunari K, Murakawa K, Yokokawa K, Horio T, Fukui T, et al. Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 1990;88:614–8.
5. Suzuki N, Matsumoto H, Kitada C, Yanagisawa M, Miyauchi T, Masaki T, et al. Immunoreactive endothelin-1 in plasma detected by a sandwich-type enzyme immunoassay. *J Cardiovasc Pharmacol* 19891;13(suppl 5):S151–2.
6. Lundberg JM, Ahlborg G, Hemsen A, Nisell H, Lunell NO, Pernow LJ, et al. Evidence for release of endothelin-1 in pigs and humans. *J Cardiovasc Pharmacol* 1991;17(suppl 7):S350–3.
7. Gray GA. Generation of endothelin. In: Gray GA, Webb DJ, eds. *Molecular biology and pharmacology of the endothelins*. Austin: Landes, R.G., 1995, pp. 13–32.
8. Rabelink TJ, Kaasjager KAH, Stroes ESG, Koomans HA. Endothelin in renal pathophysiology: From experimental to therapeutic application. *Kidney Int* 1996;50:1827–33.
9. Webb DJ. Endothelin and blood pressure regulation. *J Hum Hypertens* 1996;10:383–6.
10. Webb DJ, Monge JC, Rabelink TJ, Yanagisawa M. Endothelin: new discoveries and rapid progress in the clinic. *Trends Pharmacol Sci* 1998;19:5–8.
11. Kohno M, Yasunari K, Murakawa K, Yokokawa K, Horio T, Fukui T, Takeda T. Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 1990;88:614–18.
12. Suzuki N, Matsumoto H, Miyauchi T, Kitada C, Tsuda M, Goto K, et al. Sandwich-enzyme immunoassays for endothelin family peptides. *J Cardiovasc Pharmacol* 1991;17(suppl. 7):S420–2.
13. Yoshimoto S, Ishizaki Y, Sasaki T, Murota S. Effect of carbon dioxide and oxygen on endothelin production by cultured porcine cerebral endothelial cells. *Stroke* 1991;22:378–83.
14. Lundberg JM, Ahlborg G, Hemsen A, Nisell H, Lunell NO, Pernow J, et al. Evidence for release of endothelin-1 in pigs and humans. *J Cardiovasc Pharmacol* 1991;17(suppl. 7):S350–3.
15. Plumpton C, Haynes WG, Webb DJ, Davenport AP. Phosphoramidon inhibition of the *in vivo* conversion of big endothelin-1 to endothelin-1 in human forearm. *Br J Pharmacol* 1995;116:1821–8.

16. Plumpton C, Ferro CJ, Haynes WG, Webb DJ, Davenport AP. The increase in human plasma immunoreactive endothelin but not big endothelin-1 or its C-terminal fragment induced by systemic administration of the endothelin antagonist TAK-044. *Br J Pharmacol* 1996;119:311–14.
17. Suzuki N, Matsumoto H, Kitada C, Masaki T, Fujino M. A sensitive sandwich-enzyme immunoassay for human endothelin. *J Immunol Methods* 1989;118:245–50.
18. Suzuki N, Matsumoto H, Kitada C, Yanagisawa M, Miyauchi T, Masaki T, Fujino M. Immunoreactive endothelin-1 in plasma detected by a sandwich-type enzyme immunoassay. *J Cardiovasc Pharmacol* 1989;13(suppl. 5):S151–2.
19. Predel HG, Meyer-Lehnert H, Backer A, Stelkens H, Kramer HJ. Plasma concentrations of endothelin in patients with abnormal vascular reactivity. Effects of ergometric exercise and acute saline loading. *Life Sci* 1990;47:1837–43.
20. Shichiri M, Hirata Y, Ando K, Emori T, Ohta K, Kimoto S, et al. Plasma endothelin levels in hypertension and chronic renal failure. *Hypertension* 1990;15:493–6.
21. Vierhapper H, Wagner OF, Nowotny P, Waldhausl W. Effect of endothelin-1 in man. *Circulation* 1990;81:1415–18.
22. Veglio F, Bertello P, Pinna G, Mulatero P, Rossi A, Gurioli L, et al. Plasma endothelin in essential hypertension and diabetes mellitus. *J Hum Hypertens* 1993;7:321-5.
23. Hoffman A, Grossman E, Goldstein DS, Gill JR Jr., Keiser HR. Urinary excretion rate of endothelin-1 in patients with essential hypertension and salt sensitivity. *Kidney Int* 1994;45:556–60.
24. Letizia C, Cerci S, De Ciocchis A, D'Ambrosio C, Scuro I, Scavo D. Plasma endothelin-1 levels in normotensive and borderline hypertensive subjects during a standard cold pressor test. *J Hum Hypertens* 1995;9:903–7.
25. Zoccali C, Leonardis D, Parlongo S, Mallamaci F, Postorino M. Urinary and plasma endothelin 1 in essential hypertension and in hypertension secondary to renoparenchymal disease. *Nephrol Dial Transplant* 1995;10:1320–3.
26. Rolinski B, Sadri I, Bogner J, Goebel FD. Determination of endothelin-1 immunoreactivity in plasma, cerebrospinal fluid and urine. *Res Exp Med* 1994;194:9–24.
27. Saito Y, Nakao K, Mukoyama M, Shirakami G, Itoh H, Yamada T, et al. Application of monoclonal antibodies for endothelin to hypertensive research. *Hypertension* 1990;15:734–8.
28. Saito Y, Nakao K, Mukoyama M, Imura H. Increased plasma endothelin level in patients with essential hypertension [letter]. *New Engl J Med* 1990;322:205.
29. Naruse M, Kawana M, Hifumi S, Naruse K, Yoshihara I, Oka T, et al. Plasma immunoreactive endothelin, but not thrombomodulin, is increased in patients with essential hypertension and ischemic heart disease. *J Cardiovasc Pharmacol* 1991;17(suppl. 7):S471–4.
30. Widimsky J Jr., Horky K, Dvorakova J. Plasma endothelin-1,2 levels in mild and severe hypertension. *J Hypertens* 1991;9(suppl. 6): S194–5.
31. Fernandez-Cruz C, Martin P, Fernandez L, Sanchez J, Ibarra J, Moya J, et al. Plasma endothelin is increased in young essential hypertensives but not in elderly essential or diabetic hypertensives. *J Hypertens* 1993;11(suppl. 5):S146–7.
32. Fogari R, Tettamanti F, Mugellini A, Tettamanzi D, Lusardi P, Milani M. Endothelin in mild to moderate essential hypertension: relationship between ambulatory and clinic blood pressure values. *J Hum Hypertens* 1994;8:761–4.
33. Lemne CE, Lundeberg T, Theodorsson E, de Faire U. Increased basal concentrations of plasma endothelin in borderline hypertension. *J Hypertens* 1994;12:1069–74.
34. Januszewicz A, Lapinski M, Symonides B, Dabrowska E, Kuch-Wocial A, Trzepla E, et al. Elevated endothelin-1 plasma concentration in patients with essential hypertension. *J Cardiovasc Risk* 1994;1:81–5.
35. Ergul S, Parish DC, Puett D, Ergul A. Racial differences in plasma endothelin-1 concentrations in individuals with essential hypertension. *Hypertension* 1996;28:652–5.
36. Zaporowska-Stachowiak I, Gluszek J, Chodera A. Comparison of the serum insulin and endothelin level in patients with essential and renovascular hypertension. *J Hum Hypertens* 1997;11:795–800.
37. Davenport AP, Ashby MJ, Easton P, Ella S, Bedford J, Dickerson C, et al. A sensitive radioimmunoassay measuring endothelin-like immunoreactivity in human plasma: comparison of levels in patients with essential hypertension and normotensive control subjects. *Clin Sci* 1990;78:261–4.
38. Schiffrin EL, Thibault G. Plasma endothelin in human essential hypertension. *Am J Hypertens* 1991;4:303–8.
39. Miyauchi T, Yanagisawa M, Iida K, Ajisaka R, Suzuki N, Fujino M, et al. Age- and sex-related variation of plasma endothelin-1 concentration in normal and hypertensive subjects. *Am Heart J* 1992; 123:1092–3.
40. Baldys-Waligorska A, Szybinski Z. Plasma endothelin 1/2 levels in healthy blood donors and in hypertensive patients: clinical application. *Endocrine Regul* 1993;27:83–7.
41. Haynes WG, Hand MF, Johnstone HA, Padfield PL, Webb DJ. Direct and sympathetically mediated venoconstriction in essential hypertension. Enhanced responses to endothelin-1. *J Clin Invest* 1994;94:1359–64.
42. Sorensen SS, Egeblad M, Eiskjaer H, Madsen B, Nielsen CB, Sihm I, Pedersen EB. Endothelin in renovascular and essential hypertension. *Blood Press* 1994;3:364–9.
43. Ferri C, Bellini C, Desideri G, Di Francesco L, Baldoncini R, Santucci A, De Mattia G. Plasma endothelin-1 levels in obese hypertensive and normotensive men. *Diabetes* 1995;44:431–6.
44. Schiffrin EL, Deng LY, Sventek P, Day R. Enhanced expression of endothelin-1 gene in resistance arteris in severe human essential hypertension. *J Hypertens* 1997;15:57–63.
45. Widimsky J Jr., Dvorakova J, Hladovec J, Kopecka J. Lack of evidence for the interaction between renin-angiotensin-aldosterone system and endothelin in vivo. *Physiol Res* 1996;45:241–3.
46. Noll G, Wenzel RR, Schneider M, Oesch V, Binggeli C, Shaw S, et al. Increased activation of sympathetic nervous system and endothelin by mental stress in normotensive offspring of hypertensive parents. *Circulation* 1996;93:866–9.
47. Rakugi H, Tabuchi Y, Nakamaru M, Nagano M, Higashimori K, Mikami H, Ogihara T. Endothelin activates the vascular renin-angiotensin system in rat mesenteric arteries. *Biochem Int* 1990; 21:867–72.
48. Marriott DB. Endothelin-1 stimulation of noradrenaline and adrenaline release from adrenal chromaffin cells. *Biochem Pharmacol* 1991;41:521–6.
49. Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. *Nature Med* 1995;1:944–9.
50. Yamane K, Miyauchi T, Suzuki N, Yuhara T, Akama T, Suzuki H, Kashiwagi H. Significance of plasma endothelin-1 levels in patients with systemic sclerosis. *J Rheumatol* 1992;19:1566–71.
51. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *New Engl J Med* 1991;325:997–1001.
52. Pittet JF, Morel DR, Hemsen A, Gunning K, Lacroix JS, Suter PM, Lundberg LM. Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. *Annals of Surgery* 1991;213:261–4.
53. Wei CM, Lerman A, Rodeheffer RJ, McGregor CG, Brandt RR, Wright S, et al. Endothelin in human congestive heart failure. *Circulation* 1994;89:1580–6.
54. Yasuda M, Kohno M, Tahara A, Itagane H, Toda I, Akioka K. Circulating immunoreactive endothelin in ischemic heart disease. *Am Heart J* 1990;119:801–6.
55. Moore K, Wendon J, Frazer M, Karani J, Williams R, Badr K. Plasma endothelin immunoreactivity in liver disease and the hepatorenal syndrome. *New Eng J Med* 1992;327:1774–8.
56. Yokokawa K, Tahara H, Kohno M, Murakawa K, Yasunari K, Nakagawa K, et al. Hypertension associated with endothelin-secreting malignant haemangioendothelioma. *Ann Int Med* 1991;114:213–5.
57. Cernacek P, Stewart DJ. Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. *Biochem Biophys Res Commun* 1989;161:562–7.
58. Horky K, Nemecek K, Widimsky J Jr., Jachymova M, Jindra A, Savlikova J, Peleska J et al. [Endothelin plasma levels in various

stages and types of arterial hypertension]. [Czech] Plazmaticka koncentrace endotelinu u ruznych stadii a typu arterialni hypertenze. *Cas Lek Cesk* 1994;133:407–10.
59. Gasic S, Wagner OF, Vierhapper H, Nowotny P, Waldhausl W. Regional haemodynamic effects and clearance of endothelin-1 in humans: renal and peripheral tissues may contribute to the overall disposal of the peptide. *J Cardiovasc Pharmacol* 1992;19:176–80.
60. Kaasjager KA, Koomans HA, Rabelink TJ. Effectiveness of enalapril versus nifedipine to antagonize blood pressure and the renal response to endothelin in humans. *Hypertension* 1995;25:620–5.
61. Kaasjager KA, Koomans HA, Rabelink TJ. Endothelin-1-induced vasopressor responses in essential hypertension. *Hypertension* 1997;30:15–21.
62. Naruse K, Naruse M, Watanabe Y, Yoshihara I, Ohsumi K, Horiuchi J, et al. Molecular form of immunoreactive endothelin in plasma and urine of normal subjects and patients with various disease states. *J Cardiovasc Pharmacol* 1991;17(suppl .7):S506–8.
63. Ferri C, Bellini C, Desideri G, Di Francesco L, De Mattia G, Santucci A. Dysfonction endotheliale dans l'hypertension arterielle sensible au sel. *Arch Mal Coeur Vaiss* 1996;89:971–4.
64. Rabelink TJ, Kaasjager KA, Boer P, Stroes EG, Braam B, Koomans HA. Effects of endothelin-1 on renal function in humans: implications for physiology and pathophysiology. *Kidney Int* 1994;46: 376–81.
65. Luscher TF, Seo B, Buhler FR. Potential role of endothelin in hypertension. *Hypertension* 1993;21:752–7.
66. Kohan DE. Autocrine role of endothelin in rat inner medullary collecting duct: inhibition of AVP-induced cAMP accumulation. *J Cardiovasc Pharmacol Suppl* 1993;22(suppl. 8):S174–9.
67. Kohan DE, Padilla E. Osmolar regulation of endothelin-1 production by rat inner medullary collecting duct. *J Clin Invest* 1993;91: 1235–40.
68. Hughes AK, Cline RC, Kohan DE. Alterations in renal endothelin-1 production in the spontaneously hypertensive rat. *Hypertension* 1992;20:666–73.
69. Orisio S, Benigni A, Bruzzi I, Corna D, Perico N, Zoja C, et al. Renal endothelin gene expression is increased in remnant kidney and correlates with disease progression. *Kidney Int* 1993;43:354–8.
70. Haynes WG, Ferro CE, O'Kane K, Somerville D, Lomax CC, Webb DJ. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in man. *Circulation* 1996;93: 1860–70.
71. Weber C, Schmitt R, Birnboeck H, Hopfgartner G, van Marle SP, Peeters PA, et al. Pharmacokinetics and pharmacodynamics of the endothelin-receptor antagonist bosentan in healthy human subjects. *Clin Pharm Ther* 1996;60:124–37.
72. Krum H, Viskoper RJ, Lacourciere Y, Budde M, Charlon V. The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. Bosentan Hypertension Investigators. *New Engl J Med* 1998;338:784–90.
73. Ferro CJ, Webb DJ. The clinical potential of endothelin receptor antagonists in cardiovascular medicine. *Drugs* 1996;51:12–27.

Letters to the Editor

Letters to the Editor will be published, if suitable, as space permits. They should not exceed 1000 words (typed double-spaced) in length and may be subject to editing or abridgment.

Endothelin Antagonists and Hypertension: A Question of Dose?

To the Editor:

We read with interest the recent report in *Hypertension* from Martin et al[1] on the effects of intra-arterial administration of the endothelin $ET_{A/B}$ receptor antagonist, SB 209670, on forearm blood flow in hypertensive subjects and matched controls. Their finding of forearm vasodilation to intra-arterial SB 209670 in healthy controls suggests a role for endothelin-1 (ET-1) in regulation of basal vascular tone and is consistent with our own work[2–4] and that of some,[4,5] but not all other groups,[6,7] with both ET_A receptor selective and $ET_{A/B}$ receptor antagonists. Also, contrary to some earlier work,[6,7] they find no difference from controls in the in vivo response of the resistance vessels of hypertensive subjects to ET receptor antagonism.

Our early intra-arterial studies were undertaken with the ET_A selective antagonist, BQ-123, at a dose of 100 nmol/min.[2] We have since undertaken pharmacodynamic and kinetic dose-ranging systemic studies with BQ-123 and find that this dose of BQ-123 has modest systemic effects, more on vascular resistance than blood pressure.[8,9] Maximum plasma concentrations of BQ-123 at 100 nmol/min were 585 ± 158 nmol/L,[8] and IC_{50} values for BQ-123 at the ET_A and ET_B receptors in vitro are 9 to 24 nmol/L and 10 to 18 000 nmol/L, respectively, depending on cell type.[10] Hence, when given locally into the forearm (blood flow $\approx$50 mL/min) rather than the systemic circulation ($\approx$5 000 mL/min), this dose of BQ-123 will achieve concentrations ($\approx$60 000 nmol/L) that may have functionally important inhibitory effects at the ET_B receptor.

On this basis, our laboratory has more recently delivered BQ-123 in forearm studies at a dose of 10 nmol/min, with which, if anything, greater effects on local blood flow have been seen.[4] This may be explained by the major role of the vascular ET_B receptor being to mediate vasodilation,[4,11] such that combined $ET_{A/B}$ inhibition may, by blocking ET_B mediated effects, attenuate the vasodilation associated with selective ET_A receptor antagonism.[4] We have also used BQ-788 intra-arterially (at 1 nmol/min) as an ET_B selective antagonist, here based on systemic studies showing that 30 nmol/min, but not 3 nmol/min, increases systemic vascular resistance.[11]

However, a key issue arises for the published body of work using intra-arterial administration of ET receptor antagonists. These investigations have generally used high doses that are likely to be both nonselective and systemically active, conditions that interfere with a clear interpretation of these studies. Cardillo and colleagues gave BQ-123 (at 100 nmol/min) and BQ-788 (at 50 nmol/min) by intra-arterial coadministration to hypertensives and controls to achieve dual ET receptor blockade.[6] However, it would now appear that both of these agents were given at systemically active doses. A similar problem of using a systemically active dose of TAK-044 may account for a rather modest effect on vascular tone in healthy subjects in one study[7] and the lesser effect of a greater dose of TAK-044 in another.[3] By giving systemic doses of the pharmacological probes, responses in the infused forearm may have been influenced directly by changes in systemic vascular resistance or indirectly by the activation of reflex neurohormonal mechanisms.[12] In this regard, there must remain some uncertainty about interpretation of work examining the role of endothelin in hypertension and other vascular diseases using drug administration via the brachial artery until these studies are repeated with doses of drugs that are demonstrably confined to a local action.

Jane Goddard
Lecturer in Renal Medicine
Clinical Research Centre
The University of Edinburgh

David J. Webb
Professor of Clinical Pharmacology
Clinical Research Centre
The University of Edinburgh
Western General Hospital
Edinburgh, Scotland
E-mail d.j.webb@ed.ac.uk

1. Martin P, Ninio D, Krum H. Effect of endothelin blockade on basal and stimulated forearm blood flow in patients with essential hypertension. *Hypertension*. 2002;39:821–824.
2. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet*. 1994;344:852–854.
3. Haynes WG, Ferro CE, O'Kane K, Somerville D, Lomax CC, Webb DJ. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in man. *Circulation*. 1996;93:1860–1870.
4. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation*. 1998;97:752–756.
5. Berrazueta JR, Bhagat K, Vallance P, MacAllister RJ. Dose-, and time-dependency of the dilator effects of the endothelin antagonist BQ-123, in the human forearm. *Br J Clin Pharmacol*. 1997;44:569–571.
6. Cardillo C, Kilcoyne CM, Waclawiw M, Cannon RO 3rd, Panza JA. Role of endothelin in the increased vascular tone of patients with essential hypertension. *Hypertension*. 1999;33:753–758.
7. Taddei S, Virdis A, Ghiadoni L, Sudano I, Notari M, Salvetti A. Vasoconstriction to endogenous endothelin-1 is increased in the peripheral circulation of patients with essential hypertension. *Circulation*. 1999;100:1680–1683.
8. Goddard J, Strachan FE, MacCallum H, Cumming AD, Rankin AJ, Webb DJ. Selective ETA endothelin receptor antagonism is as effective in reducing blood pressure and vascular resistance as combined ETA/B receptor antagonism in both healthy volunteers and patients with chronic renal failure. *Circulation*. 2000;102:II-417. Abstract.
9. Spratt JCS, Goddard J, Patel N, Strachan FE, Rankin AJ, Webb DJ. Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men. *Br J Pharmacol*. 2001;134:648–54.
10. Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami T, Ozaki S, Nagase T. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Nat Acad Sci U S A*. 1994;91:4892–4896.
11. Strachan FE, Spratt JC, Wilkinson IB, Johnston N, Gray GA, Webb DJ. Systemic blockade of the endothelin-B receptor increases peripheral vascular resistance in healthy men. *Hypertension*. 1999;33:581–585.
12. Benjamin N, Calver A, Collier J, Robinson B, Vallance P, Webb D. Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension*. 1995;25:918–923.They should not exceed 1000 words

Response

We thank Doctors Goddard and Webb for their comments regarding intra-arterial infusion of endothelin (ET) antagonists. Goddard and Webb raise a number of relevant issues. They reiterate the importance of using subsystemic doses of vasoactive substances to test hypotheses regarding direct effects on the vasculature. The key issue is how to establish that doses are indeed subsystemic. The usual approach includes careful assessment of systemic blood

pressure and heart rate responses and/or measurement of forearm blood flow and vascular resistance in the contralateral limb.[1] This is really a general issue of the technique itself and not limited to endothelin blockade as the vasoactive substance.

Specific to endothelin blockade, however, is the issue of selectivity of the receptor antagonist. Goddard and Webb suggest that doses of the ET_A-"selective" antagonist BQ-123, assumed to be subsystemic in a number of studies,[2,3] may indeed be systemically active[4] and also block ET_B-mediated vasodilation.[5] However, this still does not explain differences in vascular responses to this agent given at the same dose and at the same infusion rate over similar periods of time. We used SB209670 in our study[6] specifically because it is nonselective, and we wished to test the impact of blockade of all major ET receptor subtypes on the vasculature, between normal subjects and patients with cardiovascular disease.

Paul Martin
Henry Krum
Clinical Pharmacology Unit
Departments of Medicine and Epidemiology
& Preventive Medicine
Monash University
Alfred Hospital
Melbourne, Australia
E-mail henry.krum@med.monash.edu.au

1. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol.* 2001;52:631–646.
2. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone *Lancet.* 1994;344:852–854.
3. Cardillo C, Kilcoyne CM, Waclawiw M, Cannon RO 3rd, Panza JA. Role of endothelin in the increased vascular tone of patients with essential hypertension. *Hypertension.* 1999;33:753–758.
4. Spratt JC, Goddard J, Patel N, Strachan FE, Rankin AJ, Webb DJ. Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men. *Br J Pharmacol.* 2001;134:648–654.
5. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation.* 1998;97:752–756.
6. Martin P, Ninio D, Krum H. Effect of endothelin blockade on basal and stimulated forearm blood flow in patients with essential hypertension. *Hypertension.* 2002;39:821–824.

British Journal of Pharmacology (2001) 134, 648–654
www.nature.com/bjp

Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men

[1]James C.S. Spratt, [1]Jane Goddard, [1]Neil Patel, [1]Fiona E Strachan, [2]Andrew J. Rankin & *,[1]David J. Webb

[1]Clinical Pharmacology Unit & Research Centre, Department of Medical Sciences, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU and [2]Department of Experimental Medicine, Clinical Sciences, Pfizer Global Research & Development, Sandwich, Kent, CT13 9NJ

1 The effect on systemic haemodynamics of BQ-123, a selective endothelin A (ETA) receptor antagonist, was investigated in healthy men by giving, on separate occasions, ascending intravenous doses of 100, 300, 1000 and 3000 nmol min^{-1} BQ-123, each for 15 min, in a randomized, placebo-controlled, double-blind study. The response of forearm blood flow to brachial artery infusion of endothelin-1 (ET-1; 5 pmol min^{-1} for 90 min) was also studied using bilateral forearm plethysmography, after systemic pre-treatment, on separate occasions, with one of two doses of BQ-123 (300 and 1000 nmol min^{-1} for 15 min) or placebo.

2 Systemic BQ-123 dose-dependently decreased systemic vascular resistance ($P<0.01$ for all doses vs placebo) and mean arterial pressure ($P<0.05$ for 300 nmol min^{-1} and $P<0.01$ for 1000 and 3000 nmol min^{-1}) during the 60 min following infusion. There were concurrent increases in heart rate and cardiac index. BQ-123, when infused systemically for 15 min, appeared to reach a maximum effect at 1000 nmol min^{-1}.

3 Intra-brachial ET-1 infusion, after pre-treatment with placebo, caused a slow onset progressive forearm vasoconstriction without systemic effects. This vasoconstriction was attenuated by pre-treatment with BQ-123 at 300 nmol min^{-1} and abolished by BQ-123 at 1000 nmol min^{-1} ($P<0.01$ vs placebo).

4 These effects occurred at concentrations of BQ-123 in the plasma (510 ± 64 nmol l^{-1}) that were ETA receptor selective, and were not accompanied by an increase in plasma ET-1 that would have indicated ETB receptor blockade.

5 We conclude that ETA-mediated vascular tone contributes to the maintenance of basal systemic vascular resistance and blood pressure in healthy men.
British Journal of Pharmacology (2001) **134**, 648–654

Keywords: Endothelin; vasodilatation; BQ-123; human; systemic

Abbreviations: ET, endothelin; FBF, forearm blood flow; IC_{50}, half-maximal inhibitory concentration

Introduction

Endothelin-1, which was first identified by Yanagisawa *et al.* (1988), is a well characterized, potent and sustained vasoconstrictor and pressor agent involved in the endothelium-mediated regulation of vascular tone (Haynes & Webb, 1998). Two ET receptor subtypes have been identified at a molecular level and characterized pharmacologically in blood vessels. ETA receptors (Arai *et al.*, 1990) have higher affinity for ET-1 than ET-3, are found on vascular smooth muscle cells, and mediate vasoconstriction. ETB receptors have equal affinity for ET-1 and ET-3 (Sakurai *et al.*, 1990) and are found on vascular endothelial cells, where they mediate endothelium dependent vasodilatation (De Nucci *et al.*, 1988; Tsukahara *et al.*, 1994). ETB receptors are also present on vascular smooth muscle cells, where they may contribute to vasoconstriction (Clozel *et al.*, 1992; Reizebos *et al.*, 1994; Seo *et al.*, 1994; Tschudi & Luscher, 1994).

Local studies in human forearm resistance vessels using phosphoramidon, an endothelin converting enzyme inhibitor, and BQ-123, a selective ETA receptor antagonist, first demonstrated the importance of ET-1 in maintaining basal resistance vessel tone, in large part through an action on the ETA receptor (Haynes & Webb, 1994). These observations have since been confirmed by others (Berrazueta *et al.*, 1997; Verhaar *et al.*, 1998). Responses in the forearm resistance vessels are usually predictive of those in the systemic circulation (Webb, 1995), so these data suggested that systemic ETA receptor antagonism would produce systemic vasodilatation. Recently, however, acute systemic administration of the selective ETA antagonist, BQ-123, was reported to have no effect on systemic haemodynamics (Schmetterer *et al.*, 1998; Montanari *et al.*, 2000).

*Author for correspondence; E-mail: j.goddard@ed.ac.uk

We hypothesized that a systemic haemodynamic effect of ETA receptor blockade was not seen in these studies because the doses of BQ-123 used provided insufficient ETA receptor blockade to affect blood pressure. In addition, because healthy subjects have a number of reflex mechanisms that serve to defend blood pressure, we hypothesized that an important effect on systemic vascular resistance might have been missed by measurement of blood pressure alone. We also hypothesized that systemically effective ETA antagonism with BQ-123 might be associated with inhibition of the vasoconstriction to exogenous doses of infused ET-1 sufficient to cause modest effects on forearm vascular resistance. We, therefore, undertook two studies. First, we assessed the haemodynamic effects of increasing doses of BQ-123, using bioimpedance cardiography, with the aim of achieving a high degree of ETA selective receptor blockade. Second, we examined whether haemodynamically active doses of BQ-123 would antagonize the response to exogenous ET-1, by infusion of local doses of ET-1 into the forearm circulation, after administration of BQ-123 systemically, and measuring responses using forearm plethysmography.

Methods

Subjects

Five healthy men (age range 18–30 years) were recruited to each of the two studies, which were performed in the Clinical Research Centre at the Western General Hospital, Edinburgh, with the approval of the local research ethics committee and the written informed consent of each subject. The investigations conformed to the principles outlined in the Declaration of Helsinki. No subject received vasoactive medication in the week before each phase of the study, and all subjects were asked to abstain from alcohol, nicotine and caffeine-containing products for 24 h and from food for at least 4 h before any measurements were made. All studies were performed from 08.30 h, in a quiet room kept at a controlled temperature (22–24°C).

Drugs

BQ-123 (Clinalfa AG, Laufelfingen, Switzerland, molecular weight 632.7) at doses ranging from 100–3000 nmol min^{-1} was used as a selective ETA receptor antagonist, with 2500 lower affinity for the ETB receptor (IC_{50} ETA of 7.3 nM against IC_{50} ETB of 18 μM) (Ihara *et al.*, 1992). The dose range was selected from previous studies investigating the local effects of BQ-123, which suggested that 100 nmol min^{-1} is the threshold around which systemic effects might be observed (Haynes & Webb, 1994). BQ-123 was dissolved in physiological saline (0.9%, Baxter Healthcare Ltd, Thetford, U.K.). Saline was also used as placebo. BQ-123 and placebo were administered in a double-blind manner and infused intravenously at a constant rate of 1 ml min^{-1} for 15 min *via* an 18 standard wire gauge (SWG) cannula sited in the right antecubital fossa (Study 1) or right distal forearm (Study 2).

ET-1 (Clinalfa AG, molecular weight 2492) was dissolved in physiological saline to a concentration of 5 pmol min^{-1} (Haynes & Webb, 1994) and infused into the left brachial artery *via* a 27 SWG steel cannula at a constant rate of 1 ml min^{-1} for a total of 90 min.

All solutions were prepared from sterile stock solutions on the day of the study. For the forearm studies 1% lignocaine (Astra Pharmaceuticals, Stockholm, Sweden) was used as a local anaesthetic.

Measurements

Systemic haemodynamics Haemodynamic measurements were made at 10 min intervals from 30 min pre-dose until 60 min post-dose, then at 30 min intervals until 2 h, then hourly until 4 h post-dose. Blood pressure was recorded in duplicate at each time-point using a well-validated semi-automated non-invasive oscillometric sphygmomanometer (Takeda UA-751 sphygmomanometer, Takeda Medical Inc) (Wiinberg *et al.*, 1988), in the right arm. Cardiac output (CO) and heart rate (HR) were recorded using a well validated non-invasive bioimpedance technique as previously described (NCCOM 3; BoMed Medical Manufacturer Ltd) (Goldstein *et al.*, 1986).

Forearm blood flow Blood flow was measured in both forearms by venous occlusion plethysmography using mercury-in-silastic gauges (Benjamin *et al.*, 1995; Webb, 1995) that were securely applied to the widest part of each forearm. The hands were excluded from the circulation during each measurement period by inflation of a wrist cuff to 220 mmHg. Upper arm cuffs were intermittently inflated to 40 mmHg for 10 in every 15 s to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. Recordings of forearm blood flow were made repeatedly at 10 min intervals over 3-min periods. Voltage output from a dual-channel Vasculab SPG 16 strain gauge plethysmograph (Medasonics Inc) was transferred to a Macintosh personal computer (Performa 475, Apple Computer Inc, Cupertino, CA, U.S.A.) using a MacLab analogue digital converter and Chart software (v. 3.2.8; both from AD Instruments, Castle Hill, NSW, Australia). Calibration was achieved using the internal standard of the Vasculab plethysmography units.

Plasma ET-1, big ET-1 and BQ-123 During Study 1, 10 ml of venous blood was obtained from a cannula inserted in the left antecubital vein before and at 5, 15 and 240 min after BQ-123 infusion for measurement of plasma ET-1 and big ET-1. In addition, during the 300 and 1000 nmol min^{-1} phases, sub-aliquots of the samples were used for plasma BQ-123 assay. Samples were collected into sterile EDTA tubes (K3 EDTA, Vacutainer, Becton Dickinson) centrifuged at $2000 \times g$ for 20 min and stored in plain tubes at −80°C prior to assay. Plasma ET-1 and big ET-1 concentrations were determined by standard radioimmunoassay (Peninsula Laboratories), as previously described (Newby *et al.*, 1998a).

BQ-123 assay BQ-123 concentrations in plasma were measured by high performance liquid chromatography (HPLC) with fluorescence detection. One volume of plasma was precipitated with 4 volumes of ethanol, ultracentrifuged at 4°C for 15 min at $10{,}000 \times g$, and the resulting supernatant injected into the HPLC column. The HPLC system consisted

of a Waters 510 HPLC pump, WISP (Waters Intelligent Sample Processor) and Spherisorb S5 ODS column (Waters Ltd, Watford, Herts. U.K.) with detection by an LS-5 fluorometric detector (Perkin-Elmer Ltd, Beaconsfield, Bucks, U.K.), with excitation and emission wavelengths of 284 and 348 nm respectively. The mobile phase consisted of 60:40 acetonitrile: de-ionized water with tri-fluoroacetic acid at a concentration of 0.1%. The peptide TAK-044 was found to fluoresce at identical wavelengths to BQ-123 and was eluted from the column with a retention time similar to but not identical with BQ-123, allowing its separate measurement. Hence, TAK-044 was used as a standard in this assay. Recovery of BQ-123 from plasma was found to be 107% and the intra- and inter-assay variations were 5.8 and 9.6% respectively.

Study design

Study 1: Systemic haemodynamic study This was a double-blind, placebo-controlled, balanced, 5-way crossover study in five subjects, investigating the responses to four doses of BQ-123 (100, 300, 1000 and 3000 nmol min^{-1}) and placebo (0.9% saline). An ascending dose regimen was followed, allowing safety and tolerability of lower doses to be assessed before proceeding. Total doses of BQ-123 administered were 1.5, 4.5, 15 and 45 μmol (or 0.95, 2.84, 9.5 and 28.4 mg). The order of the placebo dose was randomly allocated so that each subject received it on a different visit. Each visit was separated by at least 5 days. Subjects rested supine for 20 min before any haemodynamic measurements, and baseline measures were then made in the 30 min before study drug administration.

Study 2: ET-1 challenge study This was a double-blind, placebo-controlled, 3-way crossover study in five subjects (three of whom participated in the systemic study), investigating the effects of intra-arterial ET-1 on forearm blood flow (FBF), after treatment with either 300 or 1000 nmol min^{-1} of BQ-123 or placebo. After baseline infusion of saline for 30 min, subjects received a 15 min intravenous infusion of BQ-123 (300 or 1000 nmol min^{-1}) or placebo *via* a cannula in the right forearm, followed immediately by an intra-arterial infusion of ET-1 at a dose of 5 pmol min^{-1} for a total of 90 min *via* a left brachial artery cannula.

Data analysis

Study 1: Systemic haemodynamics Data were stored and analysed using the Microsoft Excel data analysis package (Excel 5.0, Microsoft Ltd). Blood pressure data at each time point were calculated as the mean of two recordings and represented as mean arterial pressure (MAP), calculated as diastolic BP + 1/3 pulse pressure. Bioimpedance data at each time point were calculated as the mean of four recordings. Data were corrected using body surface area to give cardiac index (CI) for direct comparison of subjects. Systemic vascular resistance index (SVRI) was calculated by dividing MAP by CI and expressed in arbitrary units. Baseline data were calculated as the mean of −10 and 0 min recordings. Haemodynamic data are expressed as placebo-corrected percentage change from baseline ± s.e.mean. Statistical analysis was performed on untransformed data. Responses were examined by repeated measures analysis of variance (ANOVA) and Bonferroni correction was applied to examine significance at each time point. Statistical significance was taken at the 5% level.

Using MAP & SVRI measurements from a previous placebo-controlled study over 4 h (Strachan *et al.*, 1999), the study was calculated to have a power of ~90% to detect a 15% change in MAP and 20% change in SVRI ($P=0.05$) with five subjects. The number of subjects was agreed with the local ethics committee on that basis.

Study 2: ET-1 challenge Plethysmographic data listings were extracted from the chart data files and forearm blood flows calculated for individual venous occlusion cuff inflations using a template spreadsheet (Excel 4.0; Microsoft Ltd). As flow only stabilizes after 60 s of wrist cuff inflation, recordings made in the first 60 s were not used for analysis. The last five flow recordings in each measurement period were calculated and averaged for the infused and non-infused arms (Webb, 1995). To reduce the variability of blood flow data, the ratio of flows in the two arms was calculated for each time point, in effect using the non-infused arm as a contemporaneous control for the active treatment arm (Benjamin *et al.*, 1995). Forearm blood flow results are shown as the percentage change from basal values in the ratio of blood flow between infused and non-infused arm. Data were examined by repeated measures analysis of variance (ANOVA) and Bonferroni correction was applied to examine significance at each time point. Statistical significance was taken at the 5% level.

From FBF measurements in a previous study using ET-1 at 5 pmol min^{-1} (Newby *et al.*, 1998b), the study was calculated to have a power of 99% to detect abolition of the vasoconstriction response to ET-1 by BQ-123, and a power of ~80% to detect a 66% attenuation of this response ($P=0.05$) with five subjects.

Results

Study 1: Systemic haemodynamics

All five subjects (mean age 26 ± 2 years) completed all parts of the study. No adverse effects of treatment were reported.

Plasma ET-1 and big ET-1 Baseline values of plasma ET-1 and big ET-1 concentrations ranged from 4.4 to 5.2 pg ml^{-1} and 25 to 42 pg ml^{-1} respectively. There were no significant differences between baseline plasma ET-1 or big ET-1 concentrations in any phase of the study. Neither ET-1 nor big ET-1 changed significantly following treatment with any dose of BQ-123 or placebo (Table 1A,B).

Plasma BQ-123 concentrations Plasma concentrations of BQ-123 were undetectable with both doses at baseline. For 300 nmol min^{-1} BQ-123, mean plasma concentrations were 126 ± 11 nmol l^{-1} at 5 min rising to 174 ± 20 nmol l^{-1} at 15 min. For 1000 nmol min^{-1} BQ-123, they were 424 ± 33 nmol l^{-1} and 510 ± 64 nmol l^{-1} respectively (ETA receptor IC_{50} 7.3 nM; ETB receptor IC_{50} 18 μM) (Ihara *et al.*,

Table 1 (A) Plasma ET-1 (pg ml^{-1}) levels at baseline, 5, 15 and 240 min following infusion of BQ-123. (B) Plasma big ET-1 (pg ml^{-1}) levels at baseline, 5, 15 and 240 min following infusion of BQ-123

BQ-123 (nmol min^{-1})	*Baseline* (pg ml^{-1})	*5 min* (pg ml^{-1})	*15 min* (pg ml^{-1})	*240 min* (pg ml^{-1})
(A)				
Placebo	4.91±0.69	5.16±0.74	5.81±0.50	5.77±0.75
100	4.57±0.48	4.08±0.39	3.95±0.65	4.44±0.29
300	5.23±0.52	5.29±0.49	5.49±0.95	5.88±0.65
1000	4.43±0.28	6.07±0.49	5.56±0.95	5.80±0.65
3000	2.88±0.10	4.08±0.49	4.03±0.63	4.22±0.41
(B)				
Placebo	45.7±14.4	49.3±10.7	66.2±14.4	38.8±7.1
100	37.8±7.4	29.2±1.9	26.0±2.4	35.8±5.2
300	40.2±6.5	38.5±6.3	49.5±14.1	48.4±6.0
1000	57.5±9.1	51.9±8.5	51.4±9.4	51.5±9.2
3000	26.6±6.7	34.7±0.6	30.7±3.1	30.1±4.6

Table 2 Baseline data – absolute values

BQ-123 (nmol min^{-1})	*MAP* (mmHg)	*SVRI*	*CI* (L min^{-1} m^{-2})	*HR* (b.p.m.)
Placebo	78.9±1.5	22.7±1.3	3.52±0.21	64.2±6.6
100	79.2±2.3	21.9±1.7	3.69±0.25	57.1±5.2
300	80.8±3.5	21.8±1.8	3.80±0.31	63.9±6.6
1000	78.2±2.1	22.5±2.9	3.63±0.38	54.5±4.8
3000	78.6±3.3	23.5±2.2	3.45±0.35	57.3±5.9
ANOVA				
Baseline data	$P=0.96$	$P=0.98$	$P=0.89$	$P=0.69$

1992). BQ-123 was no longer detectable in the plasma by 4 h at either dose.

Haemodynamic parameters Baseline measurements for haemodynamic parameters were similar during all treatment periods (Table 2). After BQ-123 administration, changes were apparent in all parameters by the first measurement at 10 min. Maximal changes occurred between 40 and 60 min, with a prolonged effect occurring at the two highest doses, excepting changes of heart rate, which were maximal at 15 min.

MAP decreased in a dose-dependent fashion. This was statistically significant at 300, 1000 and 3000 nmol min^{-1} BQ-123 (300 nmol min^{-1}: ANOVA $P<0.05$ vs placebo, 1000 and 3000 nmol min^{-1}: $P<0.01$ vs placebo) with a maximum mean placebo-corrected reduction of 12.4±3.5% after 3000 nmol min^{-1}. Placebo corrected SVRI also decreased in a dose dependent fashion. This decrease was significant for all doses of BQ-123 (ANOVA $P<0.01$ vs placebo). The maximum decrease in SVRI (23.3±4.3%) occurred with 3000 nmol min^{-1} of BQ-123 (Table 3 and Figure 1).

CI and HR increased significantly at all doses ($P<0.01$; ANOVA, Table 3 and Figure 1).

Study 2: ET-1 challenge

Subjects who received placebo followed by local infusion of ET-1 developed a slow onset progressive vasoconstriction in the infused arm compared to the non-infused arm (maximum reduction in FBF: −48±10% at 90 min). This response was attenuated by 300 nmol min^{-1} BQ-123 (−27±8% at 90 min $P>0.5$ vs placebo) and abolished by 1000 nmol min^{-1} BQ-123 (−8%±3%, $P<0.01$ vs placebo, Figure 2).

Discussion

We have shown, in healthy humans, that BQ-123 causes substantial systemic vasodilatation, associated with a small but significant reduction in arterial blood pressure. A dose-dependent effect was observed, with little additional effect occurring above 1000 nmol min^{-1}. This work confirms the importance of the endothelin system, and of the vascular ETA receptor in controlling vascular tone and blood pressure (Haynes & Webb, 1998), and is in accord with the results of previous local forearm infusion studies (Haynes & Webb, 1994; Berrazueta *et al*., 1997; Verhaar *et al*., 1998).

We have several reasons for concluding that the effects on vascular tone and blood pressure are mediated by the ETA receptor. Measured BQ-123 concentrations in plasma at both 300 and 1000 nmol min^{-1} were substantially greater than the IC_{50} for BQ-123 at the ETA receptor. Even so, at 1000 nmol min^{-1} the plasma concentration of 510 nmol l^{-1}, was more than 35 fold lower than the IC_{50} for the ETB receptor (18 μM), consistent with effective but selective ETA receptor blockade (Ihara *et al*., 1992). In addition, there is a substantial body of evidence that the ETB receptor is a clearance receptor for ET-1 (Fukuroda *et al*., 1994; Ozaki *et al*., 1995; Dupuis *et al*., 1996) and that agents that block the ETB receptor *in vivo* cause increases in plasma ET-1 concentrations (Haynes *et al*., 1996; Weber *et al*., 1996; Sutsch *et al*., 1998; Strachan *et al*., 1999). In contrast, in this

Table 3 Haemodynamic changes after BA-123 administration

BQ-123 (nmol min^{-1})	*MAP* (mmHg)	*SVRI*	*CI* (L min^{-1} m^{-2})	*HR* (b.p.m.)
100	−4.8±2.6%	−15.8±7.6%	14.3±8.9%	23.8±13.4%†
	(−4.0±2.5)	(−3.9±2.1)	(0.49±0.32)	(12.1±6.5)
300	−6.8±3.6%	−20.6±3.0%*	13.0±2.2%*	11.8±4.4%
	(−5.4±2.8)	(−4.5±0.7)	(0.47±0.07)	(6.8±2.4)
1000	−8.2±3.1%†	−22.7±5.2%†	17.9±5.7%†	20.0±5.9%†
	(−6.4±2.4)	(−5.4±1.9)	(0.59±0.16)	(11.6±3.0)
3000	−12.4±3.5%†	−23.3±4.3%†	12.7±1.6%	18.7±6.8%*
	(−10.1±3.2)	(−5.6±2.6)	(0.43±0.04)	(10.7±3.1)

Results given are maximum placebo corrected percentage change from baseline±s.e.mean. *$P>0.05$ vs placebo, †$P>0.01$ vs placebo: ANOVA+Bonferroni correction.

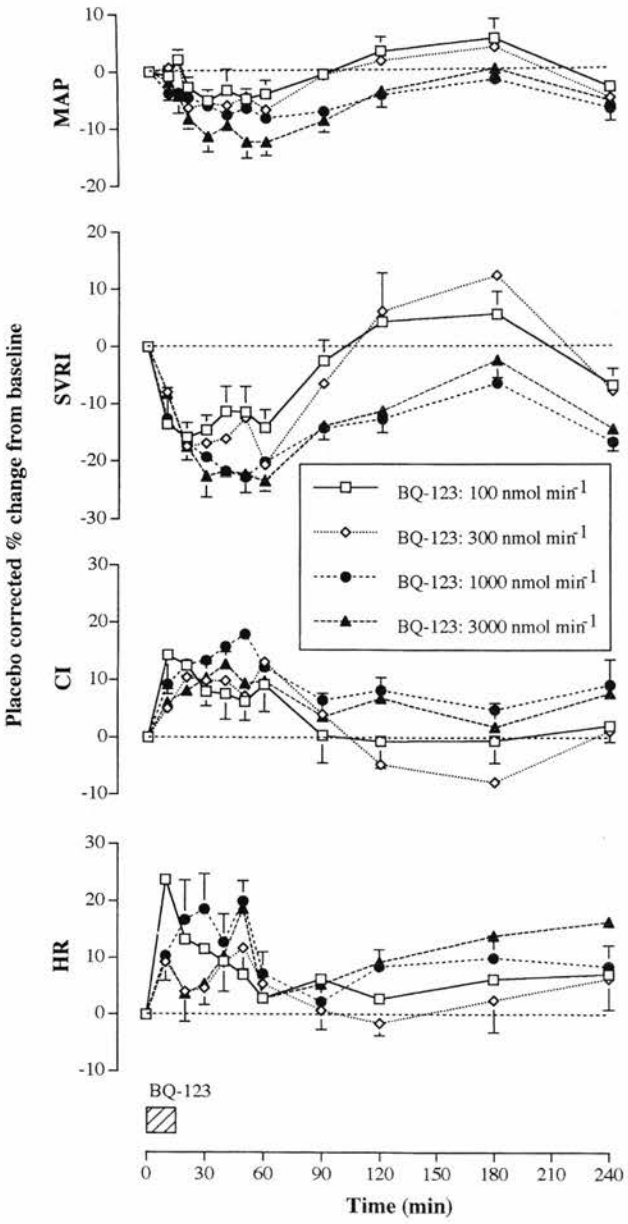


Figure 1 Change in MAP against time for ascending doses of BQ-123; change in SVRI against time for ascending doses of BQ-123; change in CI against time for ascending doses of BQ-123; change in HR against time for ascending doses of BQ-123.

study, there was no significant increase in either big ET or ET-1 plasma concentration at any dose of BQ-123 (Table 1A,B). Finally, we have previously shown that the net effect of systemic ETB receptor blockade is to cause systemic vasoconstriction, and therefore, we would anticipate that any ETB blockade would attenuate the vasodilatation associated with ETA blockade. Indeed, this effect may contribute to the lack of further vasodilatation at the highest dose of BQ-123, which was associated with a tendency for a rise in plasma ET-1 concentration, also consistent with a threshold effect on the ETB receptor at this dose.

Doses sufficient to lower blood pressure (300 and 1000 nmol min^{-1}) also antagonized the forearm vasoconstriction to brachial artery administration of ET-1 and, in keeping with its submaximal effect on SVRI, the lower dose of BQ-123 (300 nmol min^{-1}) only partially antagonized forearm vasoconstriction to ET-1. Of note, however, in the presence of a higher degree of ETA blockade, exogenous ET-1 failed to produce vasodilatation. This possibly reflects the local balance of dilator and constrictor effects mediated by endothelial and vascular smooth muscle ETB receptors. However it is also possible that the locally administered ET-1 was washed out by an increase in forearm blood flow consequent upon the vasodilatation induced by systemic BQ-123. Comparison with a constrictor agent unaffected by ETA antagonism would be needed to clarify this further.

Previous studies in healthy men (Schmetterer *et al.*, 1998; Montanari *et al.*, 2000) failed to demonstrate a significant effect of BQ-123 on basal haemodynamics. However, the doses used were substantially lower, at 23.7 nmol min^{-1} for 60 min, followed by 94.8 nmol min^{-1} for 60 min (Schmetterer *et al.*, 1998) and ~9 nmol min^{-1} for 90 min (Montanari *et al.*, 2000). These should be compared with 100 nmol min^{-1} BQ-123 for 15 min as the threshold dose for a systemic haemodynamic effect in our studies. In addition, both other studies measured blood pressure but not systemic vascular resistance, whereas, from the current study, the latter was a more powerful measure of the vascular effect of BQ-123, underlining the importance of this measurement in detecting modest haemodynamic influences. In this regard, other published studies in humans with endothelin antagonists do appear to show modest (~10 mmHg) reductions in blood pressure, with both bosentan (mixed ETA/ETB) (Weber *et al.*, 1996) and ABT-627 (ETA selective) (Verhaar *et al.*, 2000). In the latter study, although systemic haemodynamics were only recorded at 30 min and 8 h after dosing, systemic

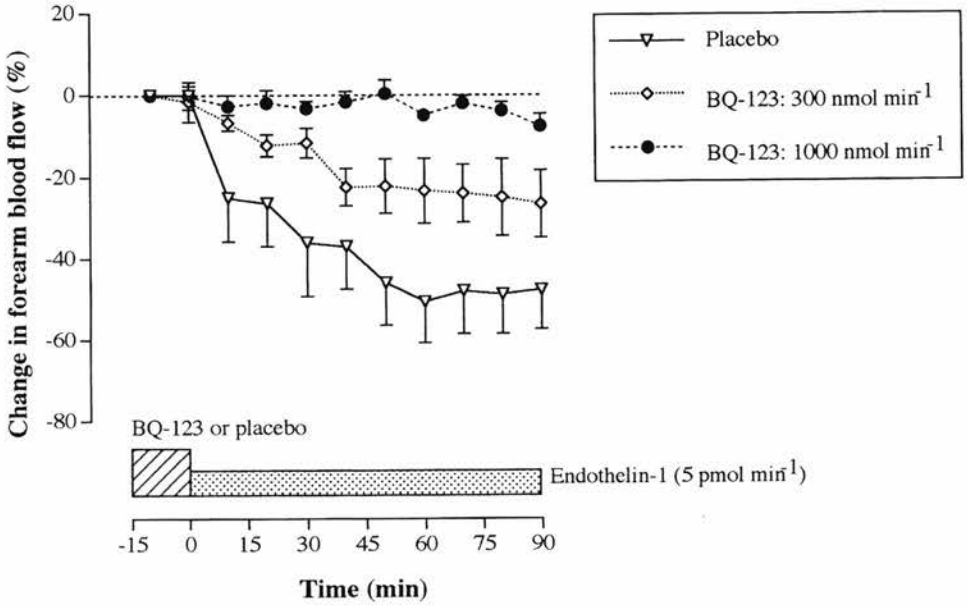


Figure 2 Graph illustrating response in forearm blood flow to intra-arterial ET-1, when pre-treated with placebo or BQ-123 (either 300 or 1000 nmol min^{-1} for 15 min).

vascular resistance was measured and significant effects on this parameter were found in both acute and chronic dosing.

It should be noted that we have also previously failed to detect significant systemic effects, as measured by blood pressure and heart rate, at our lowest dose of BQ-123 (100 nmol min^{-1}) when administered into the brachial artery (Haynes & Webb, 1994). These current results suggest that 100 nmol min^{-1} does have a small systemic action, most apparent in its effects on systemic vascular resistance, that was not detected in forearm studies, perhaps because the major vasodilatation is in other vascular beds. In recognition of this potential problem, we have more recently used a 10 fold lower dose of 10 nmol min^{-1} BQ-123, (Verhaar *et al.*, 1998), as a local dose for forearm studies. The current study confirms the rationale for this approach.

It is also interesting to note that a 15 min infusion of BQ-123 produces haemodynamic effects for up to 4 h at the higher doses. Although we have plasma estimations of BQ-123 concentrations only at 0, 15 and 240 min in the current study, subsequent experiments, with identical dosing schedules of BQ-123, demonstrate that the peak concentration is achieved at the end of the infusion, falls to $\sim 10\%$ by 30 min and is undetectable by 75 min post infusion (unpublished data). This suggests that the observed responses are a pharmacodynamic effect rather that a reflection of the plasma half-life of BQ-123. This is similar to our experience with the non-selective ET antagonist TAK 044 where the systemic haemodynamic effects of a 15 min bolus were still observable at 24 h, whereas the peptide had a plasma half-life of 30–60 min (Haynes *et al.*, 1996).

In the current study, there was an increase in heart rate similar to that observed in other acute studies (Weber *et al.*, 1996; Wenzel *et al.*, 1998). These effects are not generally seen in chronic dosing studies with endothelin antagonists in patients with either hypertension or heart failure (Krum *et al.*, 1998; Sutsch *et al.*, 1998). For this reason, the effects are probably mediated through the activation of a cardiopulmonary reflex response to systemic vasodilatation rather a direct chronotropic effect on the heart.

Although the total number of subjects studied was low ($n=5$), the power of the study was sufficient to allow clear conclusions to be drawn. Given the limited experience with BQ-123 at these systemic doses, there were safety reasons for keeping the number of subjects to a minimum. In this regard, it is reassuring to note that, despite substantial systemic vasodilatation, and significant lowering of the mean arterial pressure, no side effects were observed or reported by the subjects.

In conclusion, this study with BQ-123 demonstrates that systemic ETA receptor antagonism causes substantial peripheral vasodilatation and modest lowering of blood pressure, consistent with an important role for the endothelin system in the maintenance of vascular tone in man. It remains to be seen, in direct comparison between selective ETA and mixed ETA/B receptor antagonists, which of the therapeutic approaches will offer the greater haemodynamic benefit in specific clinical indications.

J.C.S. Spratt holds a High Blood Pressure Foundation Fellowship. J. Goddard is supported by an educational grant from Pfizer U.K. D.J. Webb is currently the recipient of a Research Leave Fellowship from the Wellcome Trust (Number WT0526330). We acknowledge the support of the British Heart Foundation (Project No: PG/99043) and Pfizer Global Research & Development, Sandwich, U.K.

References

ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H. & NAKANISHI, S. (1990). Cloning and expression of a cDNA encoding an endothelin receptor. *Nature*, **348**, 730–732.

BENJAMIN, N., CALVER, A., COLLIER, J., ROBINSON, B., VALLANCE, P. & WEBB, D. (1995). Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension*, **25**, 918–923.

BERRAZUETA, J.R., BHAGAT, K., VALLANCE, P. & MACALLISTER, R.J. (1997). Dose- and time-dependency of the dilator effects of the endothelin antagonist BQ-123, in the human forearm. *Br. J. Clin. Pharm.*, **44**, 569–571.

CLOZEL, M., GRAY, G.A., BREU, V., LOFFLER, B. & OSTERWALDER, R. (1992). The endothelin ETB receptor mediates both vasodilatation and vasoconstriction in vivo. *Biochem. Biophys. Res. Commun.*, **186**.

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, J.P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9797–9800.

DUPUIS, J., GORESKY, C.A. & FOURNIER, A. (1996). Pulmonary clearance of circulating endothelin-1 in dogs in vivo: Exclusive role of ET(B) receptors. *J. Applied Physiol.*, **81**, 1510–1515.

FUKURODA, T., FUJIKAWA, T., OZAKAI, S., ISHIKAWA, K., YANO, M. & NISHIKIBE, M. (1994). Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem. Biophys. Res. Commun.*, **199**, 1461–1465.

GOLDSTEIN, D.S., CANNON, R.O. & ZIMLICHMAN, R. (1986). Clinical evaluation of impedance cardiography. *Clin. Physiol.*, **6**, 235–238.

HAYNES, W.G., FERRO, C.J., O'KANE, K.P., SOMERVILLE, D., LOMAX, C.C. & WEBB, D.J. (1996). Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in man. *Circulation*, **93**, 1860–1870.

HAYNES, W.G. & WEBB, D.J. (1994). Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet*, **344**, 852–854.

HAYNES, W.G. & WEBB, D.J. (1998). Endothelin as a regulator of cardiovascular function in health and disease. *J. Hypertens.*, **16**, 1081–1098.

IHARA, M., ISHIKAWA, K., FUKURODA, T., SAEKI, T., FUNABASHI, K., FUKAMI, T., SUDA, H. & YANO, M. (1992). In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ETA receptor. *J. Cardiovasc. Pharmacol.*, **20** (Suppl 12): S11–S14.

KRUM, H., VISKOPER, R.J., LACOURCIERE, Y., BUDDE, M. & CHARLON, V. (1998). The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. Bosentan Hypertension Investigators. *New Engl. J. Med.*, **338**, 784–790.

MONTANARI, A., BIGGI, A., CARRA, N., FASOLI, E., CALZOLARI, M., CORSINI, F., PERINOTTO, P. & NOVARINI, A. (2000). Endothelin-A blockade attenuates systemic and renal hemodynamic effects of L-NAME in humans. *Hypertension*, **35**, 518–523.

NEWBY, D.E., JALAN, R., MASUMORI, S., HAYES, P.C., BOON, N.A. & WEBB, D.J. (1998a). Peripheral vascular tone in patients with cirrhosis: role of the renin-angiotensin and sympathetic nervous systems. *Cardiovasc. Res.*, **38**, 221–228.

NEWBY, D.E., FLINT, L.L., FOX, K.A., BOON, N.A. & WEBB, D.J. (1998b). Reduced responsiveness to endothelin-1 in peripheral resistance vessels of patients with syndrome X. *J. Am. Coll. Cardiol.*, **37**, 1585–1590.

OZAKI, S., OHWAKI, K., IHARA, M., FUKURODA, T., ISHIKAWA, K. & YANO, M. (1995). ETB-mediated regulation of extracellular levels of endothelin-1 in cultured human endothelial cells. *Biochem. Biophys. Res. Commun.*, **209**, 483–489.

REIZEBOS, J., WATTS, I.S. & VALLANCE, P.J.T. (1994). Endothelin receptors mediating functional responses in human small arteries and veins. *Br. J. Pharmacol.*, **111**, 609–615.

SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, H., KIMURA, S., GOTO, K. & MASAKI, T. (1990). Cloning of a cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. *Nature*, **348**, 732–735.

SCHMETTERER, L., DALLINGER, S., BOBR, B., SELENKO, N., EICHLER, H.G. & WOLZT, M. (1998). Systemic and renal effects of an ET-A receptor subtype-specific antagonist in healthy subjects. *Br. J. Pharmacol.*, **124**, 930–934.

SEO, B., OEMAR, B.S., SIEBENMANN, R., VON SEGESSER, L. & LUSCHER, T.F. (1994). Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation*, **89**, 1203–1208.

STRACHAN, F.E., SPRATT, J.C., WILKINSON, I.B., JOHNSTON, N., GRAY, G.A. & WEBB, D.J. (1999). Systemic blockade of the endothelin-B receptor increases peripheral vascular resistance in healthy men. *Hypertension*, **33**, 581–585.

SUTSCH, G., KIOWSKI, W., YAN, X.-W., HUNZIKER, P., CHRISTEN, S., STROBEL, W., KIM, J.-H., RICKENBACHER, P. & BERTEL, O. (1998). Short-term oral endothelin-receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. *Circulation*, **98**, 2262–2268.

TSCHUDI, M.R. & LUSCHER, T.F. (1994). Characterization of contractile endothelin and angiotensin receptors in human resistance arteries: evidence for two endothelin and one angiotensin receptor. *Biochem. Biophys. Res. Commun.*, **204**, 685–690.

TSUKAHARA, H., ENDE, H., MAGAZINE, H.I., BAHOU, W.F. & GOLIGORSKI, M.S. (1994). Molecular and functional characterization of the non-isopeptide-selective ETB receptor in endothelial cells: receptor coupling to nitric oxide synthase. *J. Biol. Chem.*, **269**, 21778–21785.

VERHAAR, M.C., GRAHN, A.Y., VAN WEERDT, A.W.N., HONING, M.L.H., MORRISON, P.J., YANG, Y.P., PADLEY, R.J. & RABELINK, T.J. (2000). Pharmacokinetics and pharmacodynamic effects of ABT-627, an oral ETA selective endothelin antagonist, in humans. *Br. J. Clin. Pharm.*, **49**, 562–573.

VERHAAR, M.C., STRACHAN, F.E., NEWBY, D.E., CRUDEN, N.L., KOOMANS, H.A., RABELINK, T.J. & WEBB, D.J. (1998). Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation*, **97**, 752–756.

WEBB, D.J. (1995). The pharmacology of human blood vessels *in vivo*. *J. Vasc. Res.*, **32**, 2–15.

WEBER, C., SCHMITT, R., BIRNBOECK, H., HOPFGARTNER, G., VAN MARLE, S.P., PEETERS, P.A.M., JONKMAN, J.H.G. & JONES, C.R. (1996). Pharmacokinetics and pharmacodynamics of the endothelin-receptor antagonist bosentan in healthy human subjects. *Clin. Pharmacol. Therap.*, **60**, 124–137.

WENZEL, R.R., FLEISCH, M., SHAW, S., NOLL, G., KAUFMANN, U., SCHMITT, R., JONES, C.R., CLOZEL, M., MEIER, B. & LUSCHER, T.F. (1998). Haemodynamic and coronary effects of the endothelin antagonist bosentan in patients with coronary artery disease. *Circulation*, **98**, 2235–2240.

WIINBERG, N., WALTER-LARSON, S., ERIKSEN, C. & NIELSEN, P.E. (1988). An evaluation of semi-automatic blood pressure manometers against intra-arterial blood pressure. *J. Amb. Monitor.*, **1**, 303–309.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.

(Received March 23, 2000
Revised July 19, 2001
Accepted July 25, 2001)

Endothelin-A Receptor Antagonism Reduces Blood Pressure and Increases Renal Blood Flow in Hypertensive Patients With Chronic Renal Failure

A Comparison of Selective and Combined Endothelin Receptor Blockade

Jane Goddard, MBChB, MRCP; Neil R. Johnston, MSc; Malcolm F. Hand, MD, MRCP; Allan D. Cumming, MD, FRCP; Ton J. Rabelink, MD, PhD; Andrew J. Rankin, PhD; David J. Webb, MD, FRCP

Background—Endothelin (ET) is implicated in the pathophysiology of chronic renal failure (CRF). We therefore studied the systemic and renal hemodynamic effects of ET receptor antagonists in CRF and examined differences between selective ETA, selective ETB, and combined ETA/B receptor blockade.

Methods and Results—We conducted a randomized, placebo-controlled, double-blind, 4-way crossover study comparing selective ET receptor antagonists BQ-123 (ETA) and BQ-788 (ETB), given alone and in combination, in acute studies in 8 hypertensive CRF patients and 8 matched healthy controls. BQ-123, alone and in combination with BQ-788, reduced blood pressure in CRF, particularly with BQ-123 alone (mean arterial pressure: controls $-4\pm2\%$, CRF $-13\pm2\%$, $P<0.01$ versus placebo). In CRF, in the face of this fall in blood pressure, BQ-123 substantially increased renal blood flow ($38.8\pm23.9\%$, $P<0.01$ versus placebo) and reduced renal vascular resistance ($-44.5\pm11.3\%$, $P<0.01$ versus placebo) when given alone but not when combined with BQ-788. These changes were accompanied by a reduction in effective filtration fraction. BQ-123, alone or in combination with BQ-788, had minimal effects on the renal circulation in healthy controls, and BQ-788 alone produced both systemic and renal vasoconstriction in CRF and healthy controls.

Conclusions—ETA receptor antagonism was highly effective in lowering blood pressure in CRF patients currently treated for hypertension. In addition, there were effects consistent with a renoprotective action. However, because the ETB receptor appears to play a key role in the maintenance of tonic renal vasodilation, combined ETA/B receptor antagonism, although it lowered blood pressure, did not confer these renal benefits. **(*Circulation*. 2004;109:1186-1193.)**

Key Words: blood flow ■ blood pressure ■ endothelin ■ kidney ■ hemodynamics

The endothelins are powerful vasoconstrictor peptides, of which endothelin-1 (ET-1) is the major isoform. It is produced by vascular endothelium and acts through 2 receptors[1,2] in the blood vessels to modulate vascular tone. Endothelin-A (ETA) and endothelin-B (ETB) receptors are found in vascular smooth muscle, where their activation mediates vasoconstriction.[3,4] ETB receptors are also found on vascular endothelium, where their activation promotes vasodilation through the release of nitric oxide and prostaglandins.[4] Additionally, vascular ETB receptors act as a major source of ET-1 clearance from the circulation,[5] and renal tubular ETB receptors may contribute to natriuresis.[6] Studies in humans show the importance of ET-1 in the maintenance of vascular tone[7] and blood pressure[8] and suggest therapeutic potential for endothelin receptor antagonists in pathophysiological states characterized by vasoconstriction, such as essential hypertension and pulmonary arterial hypertension.[9,10]

Chronic renal failure (CRF) is characterized by systemic and renal vasoconstriction and commonly associated with hypertension. Given that endothelin receptor antagonists cause vasodilation,[7,11] lower blood pressure,[9,11] and ameliorate renal dysfunction in experimental models of kidney disease,[12] they may be useful in the treatment of CRF. The aim of the present study was to show whether endothelin receptor antagonists would produce beneficial systemic or renal hemodynamic effects in CRF. Because of the potential opposing effects at ETA and ETB receptors, we directly compared the effects of regimens involving ETA and ETB

Received July 28, 2003; revision received November 12, 2003; accepted November 25, 2003.

From the Centre for Cardiovascular Science (J.G., N.R.J., A.D.C., D.J.W.), University of Edinburgh, Edinburgh, United Kingdom; Monklands Hospital (M.F.H.), Airdrie, Lanarkshire, United Kingdom; Department of Nephrology & Hypertension (T.J.R.), University Hospital, Utrecht, Netherlands; and Pfizer Global Research & Development (A.J.R.), Sandwich, United Kingdom.

Guest Editor for this article was Wilson S. Colucci, MD, Boston University Medical Center, Boston, Mass.

Correspondence to Professor D.J. Webb, Clinical Research Centre, Centre for Cardiovascular Science, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU, United Kingdom. E-mail d.j.webb@ed.ac.uk

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000118499.69469.51**

TABLE 1. Subject Demographic Data

	Healthy Volunteers	Renal Patients	P (*t* Test)
Age, y	47±5 (23–64)	46±5 (25–67)	NS
Body mass index, kg/m^2	26±2 (18–31)	27±1 (24–33)	NS
MAP, mm Hg	92.8±3.1 (83.0–103.6)	98.8±3.5 (78.8–109.5)	NS
Creatinine, μmol/L	85±5 (62–111)	255±41 (122–434)	<0.01
Cholesterol, mmol/L	5.3±0.3 (3.9–6.1)	5.6±0.2 (4.5–6.7)	NS
Urinary sodium excretion, mmol/24 h	136±14 (56–199)	150±14 (74–246)	NS
Urinary protein excretion, mg/24 h	0*	476±110 (n=7; 27–2033)†	<0.01

Values are mean±SEM (range).
*Values below limit of laboratory assay.
†n=7; 1 value below limit of detection.

receptor blockade separately and in combination in CRF patients and matched healthy controls.

Methods

Subjects

We aimed to recruit 8 men with stable CRF and 8 matched healthy controls to the studies, which were performed in the University of Edinburgh's Clinical Research Centre with the approval of the local research ethics committee and the written informed consent of each subject. The investigations conformed to the principles outlined in the Declaration of Helsinki.

For 3 days before each study, subjects adhered to a standard diet containing 150 mmol of sodium. All subjects abstained from alcohol, nicotine, and caffeine-containing products for 24 hours and had a light breakfast before attending on each study day. All studies were performed in a quiet, temperature-controlled room at 22°C to 24°C, with the subject recumbent throughout, except when voiding urine.

Healthy subjects who had taken any medications in the previous 2 weeks were excluded from the study. Patients continued taking their normal medication up to and including each study day with the exception of diuretics, which they omitted that morning. To enhance homogeneity and avoid other influences on vascular reactivity, patients with vasculitis, other systemic inflammatory disease, polycystic kidney disease, nephrotic syndrome, or obstructive uropathy were excluded. Additionally, patients with significant comorbid disease, including diabetes mellitus, heart or lung disease, peripheral vascular disease, or hypercholesterolemia, were excluded. The 2 study groups were matched for age, weight, serum cholesterol, and blood pressure (Table 1).

Drugs

BQ-123 (Clinalfa AG), a selective ETA receptor antagonist,[13] was infused at 100 and 1000 nmol/min for 15 minutes at each dose. These doses were selected from a previous study as having a threshold and maximum hemodynamic effect in healthy controls.[8] BQ-788 (Clinalfa), a selective ETB receptor antagonist,[14] was infused at 30 and 300 nmol/min for 15 minutes, doses shown to be hemodynamically active in a previous systemic dose-ranging study.[15] Drugs were dissolved in physiological saline (0.9%; Baxter Healthcare Ltd) and infused intravenously at a constant rate of 1 mL/min. Saline was administered as placebo.

Para-aminohippurate sodium (PAH; Clinalfa) and inutest (Fresenius Pharma, Austria GmbH) were dissolved in dextrose 5% (Baxter) and administered as a bolus loading dose of 0.4 g of PAH and 3.5 g of inutest in 100 mL of dextrose over 15 minutes and a maintenance infusion of 6.6 g/L PAH and 10 g/L inutest at a rate of 2 mL/min. For subjects with a calculated glomerular filtration rate (GFR) <40 mL/min, doses of PAH and inutest were reduced by one third.

Assays

At prespecified time points, venous blood was collected into EDTA tubes (Sarstedt) for measurement of PAH, inulin, and hematocrit (Hct), plasma ET-1, plasma renin activity, angiotensin II, and aldosterone, and into plain tubes (Sarstedt) for the measurement of serum sodium. Additionally, 20-mL aliquots of urine from each voiding were collected into plain tubes for the measurement of urinary PAH, inulin, sodium, and protein.

Hct was measured on whole blood with a Coulter counter. All other blood samples were centrifuged immediately at 1000*g* at 4°C for 20 minutes, and plasma and urine were stored in plain tubes at −80°C. Inulin was determined by spectrophotometry after hydrolysis to fructose, and PAH and BQ-123[8] were determined by high-performance liquid chromatography. BQ-788 assay was not sufficiently sensitive for its detection in plasma. Urinary and plasma sodium concentrations were measured by flame photometry. ET-1, angiotensin II, plasma renin activity, and aldosterone were determined by radioimmunoassay.[16–18]

Study Protocol

This was a randomized, double-blind, placebo-controlled study. Subjects attended for 4 visits, receiving placebo, BQ-123, BQ-788, or the combination of BQ-123 and BQ-788. Because previous studies with the same doses of BQ-123 or BQ-788 have demonstrated that hemodynamic changes return to baseline after 4 hours,[8,15] each visit was separated by ≥7 days to ensure complete washout of the study drugs. An individual otherwise unconnected with the study prepared the drugs to maintain blinding. On each study day, an 18 standard wire gauge cannula was sited in an antecubital vein in each arm. Diuresis was induced by 500 mL of 5% dextrose over 30 minutes through the left arm cannula. After 15 minutes, loading doses of PAH and inutest were administered through the same cannula. Thereafter, maintenance infusions of PAH and inutest and 5% dextrose at 260 mL/h continued throughout the study. Blood pressure, cardiac output and heart rate were recorded by validated noninvasive automated techniques every 15 minutes,[19,20] and urine was collected every 30 minutes by spontaneous voiding. After a 2-hour equilibration period, baseline measurements were made over 2 30-minute urine-collection periods. The low dose of antagonist was then administered through the right antecubital cannula, followed by 3 30-minute collection periods. The higher dose of antagonist was then administered, followed by 5 further 30-minute collection periods.

At the midpoint of each collection period, blood was sampled from the right antecubital cannula for PAH, inulin, sodium, and Hct. At 0, 60, and 90 minutes after the start of low- and high-dose antagonist and at the end of the study, additional samples were taken for plasma hormone measurements. BQ-123 was measured before and at 15, 45, and 90 minutes after the start of each dose of antagonist and at the end of the study.

TABLE 2. Renal Patients: Diagnosis and Medications

Subject	Cause of Renal Impairment	Drugs Used
1	IgA nephropathy	Enalapril
2	IgA nephropathy	Ranitidine
3	IgA nephropathy	Enalapril, doxazosin, bicarbonate, omeprazole
4	IgA nephropathy/HSP	Labetalol
5	Proliferative GN	Fosinopril, atenolol
6	Proliferative GN	Enalapril, metoprolol, nifedipine, allopurinol, frusemide
7	Renal calculi, single kidney	Enalapril, bicarbonate, 1-α calcidol
8	Not known; late presentation	Lisinopril, bicarbonate, omeprazole, frusemide

HSP indicates Henoch-Schönlein purpura; GN, glomerulonephritis.

Data Analysis

Data were stored and analyzed with Microsoft Excel (version 5.0, Microsoft Ltd). Demographic data are expressed as mean±SEM, and comparisons between groups were examined by unpaired Student's *t* tests. Blood pressure at each time point was calculated as the mean of 2 recordings and represented as mean arterial pressure (MAP=diastolic blood pressure+1/3 pulse pressure). Bioimpedance data at each time point were calculated as the mean of 4 recordings, each the average of 15 consecutive heart beats. Data were corrected with body surface area to give cardiac index for direct comparison of subjects. Systemic vascular resistance index (SVRI) was calculated by dividing MAP by cardiac index and expressed in dyne · s · m^{-2} · cm^{-5}. GFR and effective renal plasma flow (ERPF) were calculated from inulin and PAH clearances, respectively.[21] Effective renal blood flow (ERBF) was calculated by dividing ERPF by (1−Hct), effective renal vascular resistance (ERVR) by dividing MAP by ERBF, and effective filtration fraction (EFF) by dividing GFR by ERPF×100%. Urinary sodium excretion was calculated as urinary sodium×urinary flow rate and fractional excretion as urinary sodium×plasma inulin divided by plasma sodium×urine inulin.

Baseline data were calculated as the mean of the 2 time points that immediately preceded administration of the first study drug. Hemodynamic results are expressed as mean±SEM placebo-corrected maximum change from baseline. Statistical analysis was performed on untransformed data. Four comparisons of interest were preidentified as placebo versus BQ-123, versus BQ-788, and versus BQ-123/788, and BQ-123 versus BQ-123/788. Responses were examined by repeated-measures ANOVA, and Bonferroni correction was used to assess significance at specific time points. Statistical significance was taken at the 5% level.

Results

Twelve renal patients were recruited; 1 developed nausea after receiving BQ-123, and 1 was unable to void urine at 30-minute intervals. Two withdrew for reasons unrelated to the study. Eight patients and all healthy controls completed all phases of the study without adverse events. The renal diagnosis and concomitant medication taken by the patients who completed the study are given in Table 2. Baseline parameters are shown in Table 3.

Systemic Hemodynamics

In CRF patients, placebo was associated with increases in SVRI to study end (3478±240 versus 3797±301 dyne · s · m^{-2} · cm^{-5}, $P<0.05$) and MAP (100.7±3.8 versus 108.0±4.1 mm Hg, $P<0.01$), consistent with the waning effects of antihypertensive medication (Figures 1 and 2).

TABLE 3. Baseline Data

	Healthy Volunteers	Renal Patients	*P* (*t* Test)
MAP, mm Hg	94.0±2.2 (86.5–104.9)	100.5±4.0 (76.9–110.1)	NS
SVRI, dyne · s · m^{-2} · cm^{-5}	3089±269 (1558–4068)	3479±270 (2296–4478)	NS
Cardiac index, L · min^{-1} · m^{-2}	2.6±0.3 (1.9–4.6)	2.4±0.2 (2.0–3.2)	NS
Heart rate, bpm	58.2±2.3 (47.2–67.4)	56.6±1.8 (49.1–62.4)	NS
ERBF, mL/min	683±41 (534–844)	295±58 (81–571)	<0.01
ERVR, mm Hg · min^{-1} · L^{-1}	148±12 (108–191)	489±101 (208–973)	<0.01
EFF, %	25.4±2.0 (17.8–36.3)	20.8±1.8 (13.5–30.7)	NS
GFR, mL · min^{-1} · 1.73 m^{-2}	116±11 (80–164)	39±6 (19–67)	<0.01
UNaV, μmol/min	143±11 (56–215)	180±31 (70–341)	NS
Urinary protein, mg/L	0*	453±82 (101–1435)†	<0.01
Plasma ET-1, pg/mL	4.2±0.3 (2.9–5.3)	5.6±0.3 (4.6–7.3)	<0.01
PRA, pg · mL^{-1} · h^{-1}	3.6±0.5 (2.2–6.4)	10.5±2.3 (3.3–20.6)	<0.05
Plasma ANG II, pg/mL	7.7±1.0 (4.4–12.6)	7.6±1.1 (4.2–13.8)	NS
Plasma aldosterone, pg/mL	61±4 (45–81)	88±28 (33–274)	NS

NS indicates not significant; UNaV, urinary sodium excretion; PRA, plasma renin activity; and ANG II, angiotensin II .

Values are given as mean of 2 baseline periods over the 4 study days ±SEM (range).

*Values below limit of laboratory assay.

†n=7; 1 value below limit of detection.

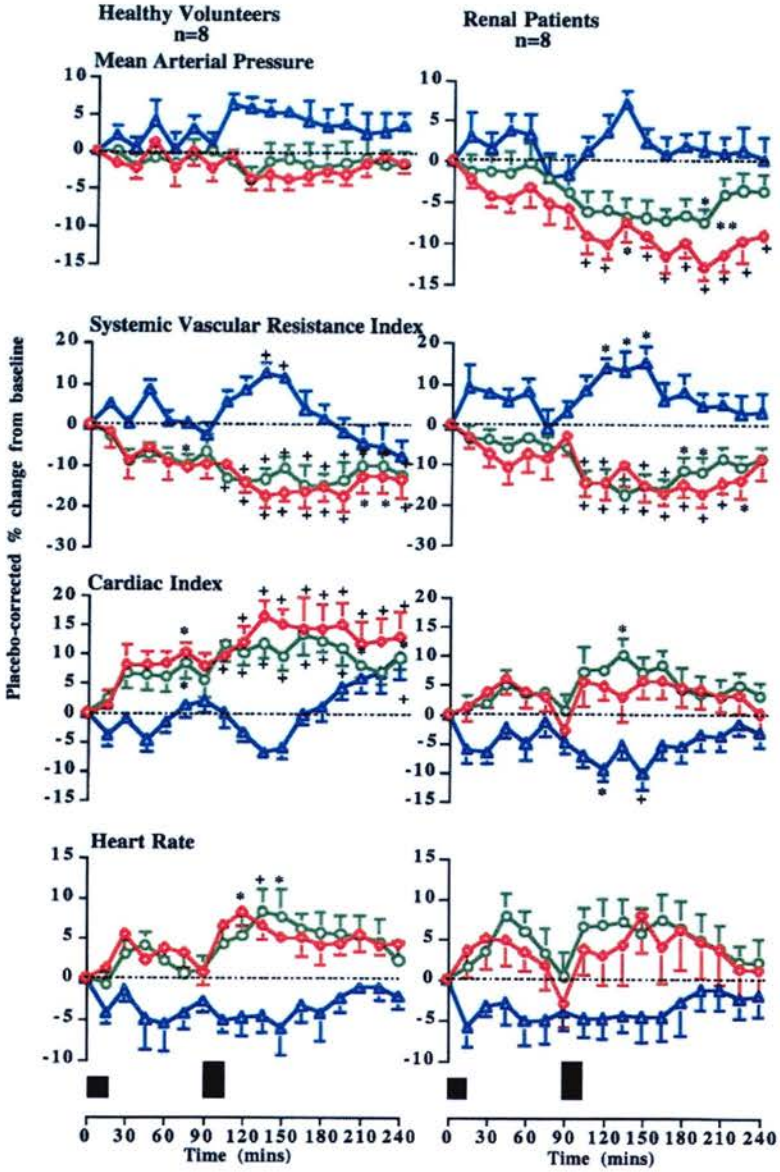


Figure 1. Systemic hemodynamics after endothelin receptor antagonism. Values are given as mean placebo-corrected % change from baseline ±SEM. Black blocks, Administration of endothelin antagonist/placebo; red diamonds, BQ-123; green circles, BQ-123/788; and blue triangles, BQ-788. *$P<0.05$ vs placebo, +$P<0.01$ vs placebo, **$P<0.05$ vs BQ-123 (ANOVA plus Bonferroni correction for significance at specific time points).

SVRI was reduced to a similar extent by BQ-123 and BQ-123/788 (BQ-123 -630 ± 145 dyne · s · m^{-2} · cm^{-5}, BQ-123/788 -617 ± 158 dyne · s · m^{-2} · cm^{-5}, both $P<0.01$ versus placebo). MAP was reduced in CRF patients after BQ-123/788 (-7.4 ± 1.6 mm Hg, $P<0.01$ versus placebo) but to a greater extent after BQ-123 alone (BQ-123 -12.9 ± 1.7 mm Hg, $P<0.01$ versus placebo and BQ-123/788). Systemic hemodynamic responses to endothelin receptor antagonism showed no correlation with baseline blood pressure.

In healthy controls, placebo did not alter systemic hemodynamics. Both BQ-123 and BQ-123/788 reduced SVRI to a similar extent, equivalent to that seen in CRF (BQ-123 -591 ± 104 dyne · s · m^{-2} · cm^{-5}, BQ-123/788 -498 ± 159 dyne · s · m^{-2} · cm^{-5}, both $P<0.01$ versus placebo). The reductions in MAP after BQ-123 and BQ-123/788 were equal (BQ-123 -3.6 ± 1.4 mm Hg, BQ-123/788 -3.7 ± 1.6 mm Hg, both $P<0.01$ versus placebo) and less than those seen in CRF ($P<0.05$). BQ-788 increased MAP (CRF 7.0 ± 1.6 mm Hg, healthy controls 5.8 ± 1.5 mm Hg, both $P<0.01$ versus placebo) and SVRI (CRF 454 ± 114 dyne · s · m^{-2} · cm^{-5}, $P<0.01$ versus placebo; healthy controls 390 ± 76 dyne · s^{-1} · m^{-2} · cm^{-5}, $P<0.05$ versus placebo) to a similar extent in CRF patients and controls.

Renal Hemodynamics

In CRF patients, BQ-123 but not BQ-123/788 produced striking increases in ERBF and reductions in ERVR and EFF (ERBF 102 ± 74 mL/min, ERVR -243 ± 91 mm Hg · min · L^{-1}, EFF $-4.2\pm2.9\%$; all $P<0.01$ versus placebo and BQ-123/788). GFR did not change. By contrast, in healthy controls, BQ-123 and BQ-123/788 were neutral with respect to ERBF, ERVR, EFF, and GFR. (See Figures 2 and 3.)

In both groups, BQ-788 reduced ERBF (CRF -77 ± 72 mL/min, healthy controls -134 ± 47 mL/min; both $P<0.05$ versus placebo) and increased ERVR (CRF 112 ± 63 mm Hg · min · L^{-1}, healthy controls 39 ± 12 mm Hg · min · L^{-1}; both $P<0.05$ versus placebo). These changes were apparent even

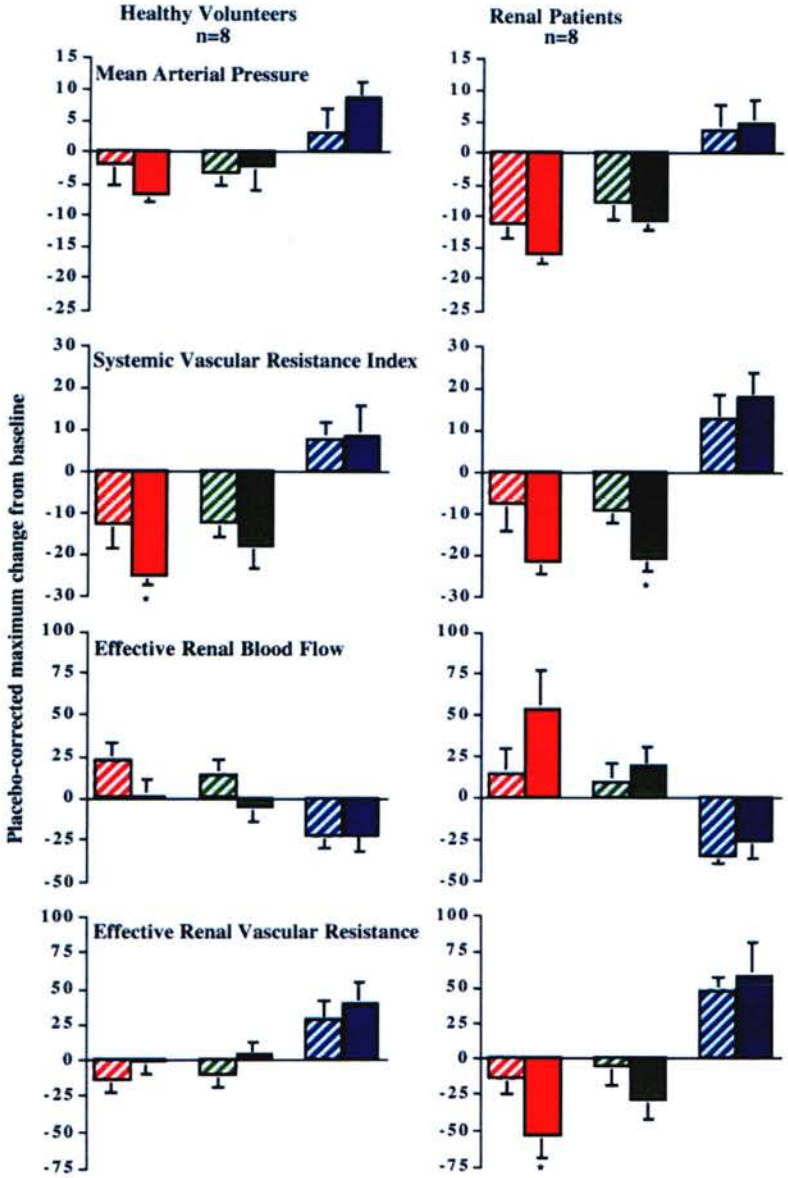


Figure 2. Maximum effects of post-endothelin receptor antagonism on systemic and renal hemodynamics. Values are given as mean of maximum placebo-corrected change from baseline ±SEM. Low dose: red hatched bars, BQ-123; green hatched bars, BQ-123/788; blue hatched bars, BQ-788. High dose: solid red bars, BQ-123; solid green bars, BQ-123/788; solid blue bars, BQ-788. *$P<0.05$ vs low dose (*t* test).

at the low dose and were associated with a reduction in GFR and an increase in EFF.

Urinary Sodium Excretion and Protein Excretion

No changes in sodium excretion or fractional excretion were observed in either CRF or healthy controls. Urinary protein excretion was undetectable for 1 CRF subject and all healthy controls. In the remaining 7 CRF patients, with measurements uncorrected for changes in GFR, BQ-788 produced a small increase in urinary protein excretion ($P<0.01$ versus placebo), but no changes were observed after BQ-123 or BQ-123/788 (Figure 4). After correction for GFR, BQ-788 and BQ-123/788 did not affect proteinuria, but BQ-123 reduced protein leak by 46% (-8.1 ± 4.9 μg/min; ANOVA $P<0.01$ versus placebo, $P<0.05$ versus BQ-123/788), an effect most apparent in subjects with higher baseline urinary protein excretion.

Plasma Hormone and BQ-123 Concentrations

Plasma ET-1 increased to a similar extent after high dose BQ-788 and BQ-123/788 but was unaltered by placebo or BQ-123 (Figure 5). Other hormones were unaffected by endothelin receptor antagonism. BQ-123 was detectable in plasma at 15 minutes after low-dose BQ-123 and at 15 and 45 minutes after high-dose BQ-123 and did not differ between CRF and healthy controls (CRF 1.52 ± 0.36 pg/mL, healthy controls 1.21 ± 0.17 pg/mL at 15 minutes after high dose). BQ-123 concentrations were unaffected by coadministration of BQ-788 (CRF 1.62 ± 0.20 pg/mL, healthy controls 1.26 ± 0.20 pg/mL).

Discussion

This is the first clinical study to directly compare ETA, ETB, and combined ETA and ETB receptor antagonism at systemic doses in humans. We have shown in CRF patients that selective ETA receptor antagonism produces substantial re-

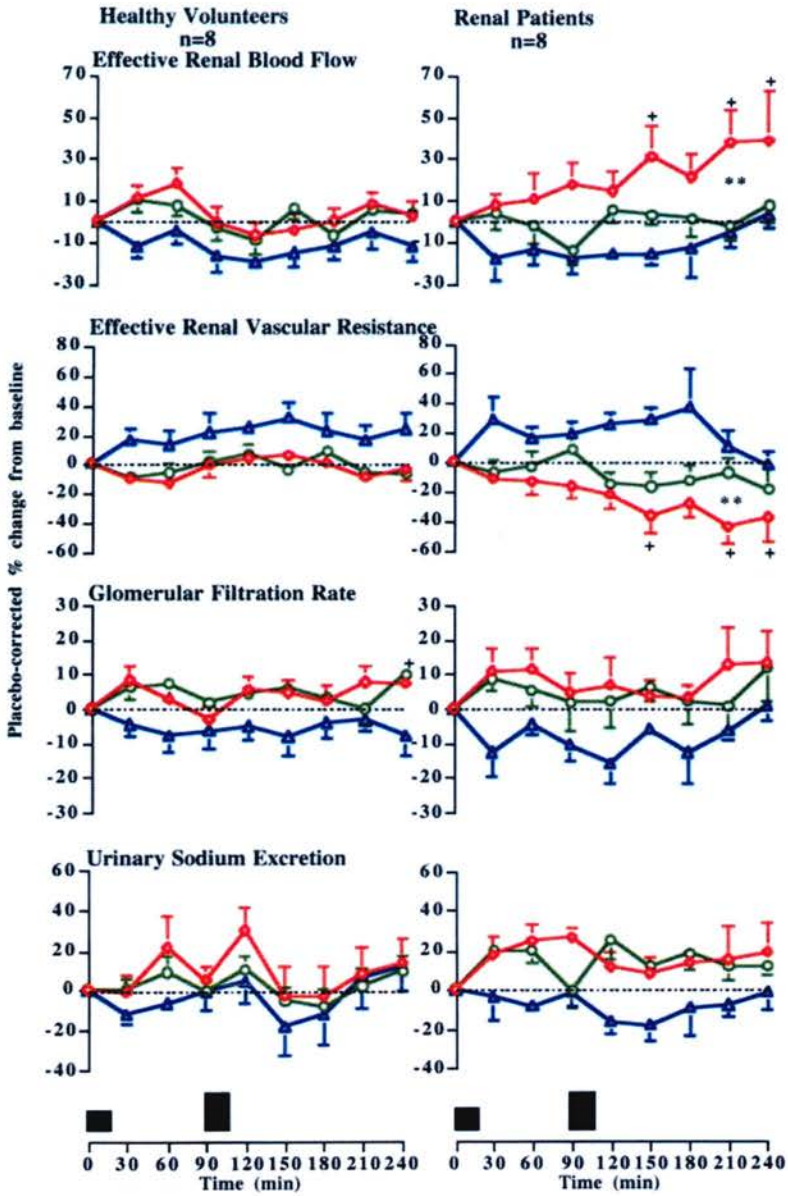


Figure 3. Renal hemodynamics after endothelin receptor antagonism. Values are given as mean placebo-corrected % change from baseline ±SEM. Black blocks, Administration of endothelin antagonist/placebo; red diamonds, BQ-123; green circles, BQ-123/788; and blue triangles, BQ-788. $^{+}P<0.01$ vs placebo; $^{**}P<0.05$ vs BQ-123 (ANOVA + Bonferroni correction for significance at specific time points).

ductions in blood pressure associated with renal vasodilation. Additionally, the reduction in filtration fraction and proteinuria in CRF patients suggests a potentially renoprotective action. Combined ETA/B receptor blockade was less effective in lowering blood pressure, had no effect on renal hemodynamics, and reduced ET-1 clearance. Selective ETB receptor antagonism alone produced substantial systemic and renal vasoconstriction. By contrast, in healthy subjects, the systemic hemodynamic effects of ETA and ETA/B receptor blockade were similar to each other but less than those seen in CRF, and there were no effects on renal hemodynamics.

These data confirm the physiological importance of ET-1, through activation of the ETA receptor, in the maintenance of basal systemic but not renal vascular resistance.[8,22] They also show that ET-1 plays a major role in regulating blood pressure and renal vascular resistance in CRF, consistent with activation of the endothelin system in this condition. The reduction in filtration fraction after ETA receptor antagonism suggests an effect primarily on efferent arteriolar tone, which may serve to reduce glomerular pressure. Consistent with our observations, an ETA receptor-selective antagonist has been shown to reduce proteinuria in type 1 diabetes.[23]

The effects of BQ-788, whether in the presence of BQ-123 or not, suggest that the net effect of ETB receptor activation on the circulation in health and renal disease is to produce vasodilation. Therefore, similar to observations in healthy subjects[15,24] and patients with heart failure,[25] any enhanced effects of constrictor ETB receptors that potentially exist in CRF are outweighed by ETB receptor–mediated vasodilation. Of particular interest, ETB receptor antagonism increased renal vascular resistance twice as much as systemic vascular resistance, which suggests that tonic ETB receptor–mediated renal vasodilation plays a key role in maintenance of renal vascular tone. This is likely to be of particular importance in CRF, in which baseline renal vascular resistance is high, and suggests ETA receptor antagonism and not dual ETA/B

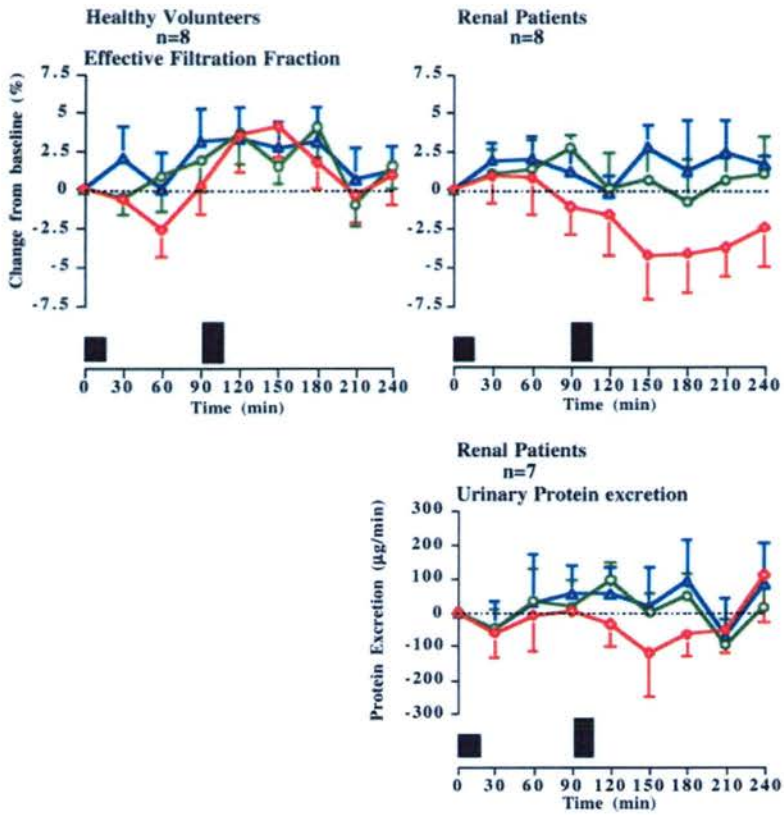


Figure 4. Effective filtration fraction (%) and urinary protein excretion (μmol/min) after endothelin receptor antagonism. Values are given as mean placebo-corrected change from baseline ±SEM. Black blocks, Administration of endothelin antagonist/placebo; red diamonds, BQ-123; green circles, BQ-123/788; and blue triangles, BQ-788.

blockade might be the best approach in renal patients. However, based on earlier work, the present study was designed to achieve effective and selective blockade of the ETA and ETB receptor[8,15,24] and not specifically to reproduce the effects of existing endothelin receptor antagonists, all of which block ETA to a greater extent than ETB receptors.[26] Therefore, although the main effects of therapeutic interest in the present study appear to derive from ETA receptor blockade and are countered by ETB blockade, drugs that cause only modest ETB receptor inhibition might produce similar effects.

Perhaps surprisingly, given the evidence for ETB receptor–mediated natriuresis shown in animal studies,[6] no changes in sodium excretion or fractional excretion were observed in the present study. However, during ETA receptor blockade, despite substantial systemic and renal vasodilation, sodium retention did not occur, which is important if these drugs are to be prescribed safely to patients with CRF.

As a limitation, the present studies were acute studies. We also studied a relatively homogeneous CRF population. Therefore, longer-term studies are now needed in a broader population of patients with kidney disease, including those with low renal perfusion pressure. In particular, because ACE inhibitors are most effective as renoprotective agents in patients with proteinuria >3 g per 24 hours, studies are needed in patients with higher degrees of urinary protein leak to address what may be a major benefit of these agents. Importantly, ethical considerations dictated that patients con-

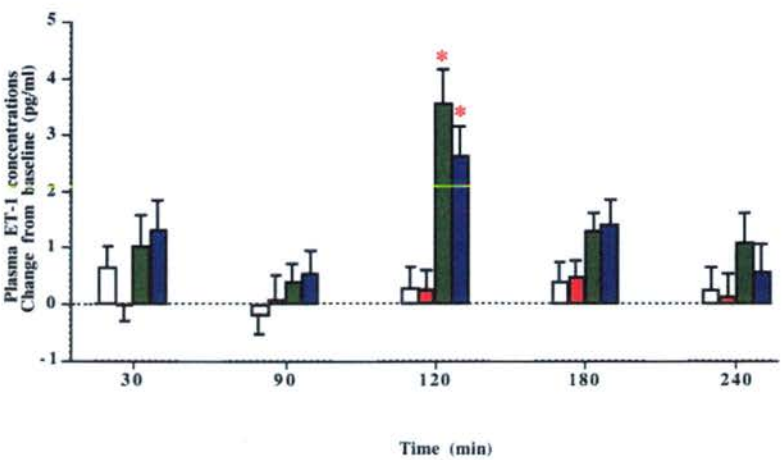


Figure 5. Plasma ET-1 concentrations (pg/mL), all subjects. Values are given as mean change from baseline ±SEM. Open bars, Placebo; solid red bars, BQ-123; solid green bars, BQ-123/788; solid blue bars, BQ-788. *$P<0.05$ vs baseline (ANOVA).

tinue their regular medications. We therefore cannot exclude an effect of prior drug therapy on our findings. However, patients with CRF generally require several drugs to control blood pressure, and we have been able to demonstrate an effect of endothelin antagonists on top of treatment that included ACE inhibitors in 6 of the 8 patients. Blood pressure and the kinetics of BQ-123 were not significantly different in CRF patients and so are unlikely to account for any differences in renal responses to endothelin antagonists between patients and controls.

In conclusion, we have shown that selective ETA receptor antagonists may be valuable antihypertensive drugs in patients with CRF as well as offering additional benefits, including renoprotection. Endothelin receptor antagonists also improve endothelial function,[27] reduce inflammation and fibrosis,[28] and reverse vascular remodeling[29] and so may offer additional benefits to renal patients, who are at high cardiovascular risk. On this basis, longer-term studies in patients with CRF are justified, with particular attention to effects on proteinuria as a surrogate marker for the progression of renal disease.

Acknowledgments

Dr Goddard was supported by an educational grant from Pfizer Global Research & Development, Sandwich, UK, and Professor Webb was the recipient of a Research Leave Fellowship from the Wellcome Trust (No. WT0526330).

References

1. Arai H, Hori S, Aramori I, et al. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature*. 1990;348:730–732.
2. Sakurai T, Yanagisawa M, Takuwa Y, et al. Cloning of a cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. *Nature*. 1990;348:732–735.
3. Moreland S, McMullen DM, Delaney CL, et al. Venous smooth muscle contains vasoconstrictor ETB-like receptors. *Biochem Biophys Res Commun*. 1992;184:100–106.
4. Gray G, Webb DJ. The endothelin system and its potential as a therapeutic target in cardiovascular disease. *Pharmacol Ther*. 1996;72: 109–148.
5. Dupuis J, Goresky CA, Fournier A. Pulmonary clearance of circulating endothelin-1 in dogs in vivo: exclusive role of ETB receptors. *J Appl Physiol*. 1996;81:1510–1515.
6. Kohan DE. Endothelins in the normal and diseased kidney. *Am J Kidney Dis*. 1997;29:2–26.
7. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet*. 1994;344:852–854.
8. Spratt JCS, Goddard J, Patel N, et al. Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men. *Br J Pharmacol*. 2001;134:648–654.
9. Krum H, Viskoper RJ, Lacourciere Y, et al. The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. *N Engl J Med*. 1998;338:784–790.
10. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med*. 2002;346:896–903.
11. Haynes WG, Ferro CE, O'Kane K, et al. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in man. *Circulation*. 1996;93:1860–1870.
12. Benigni A, Remuzzi G. Endothelin antagonists. *Lancet*. 1999;353: 133–138.
13. Ihara M, Ishikawa K, Fukuroda T, et al. In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ETA receptor. *J Cardiovasc Pharmacol*. 1992;20(suppl 12):S11–S14.
14. Ishikawa K, Ihara M, Noguchi K, et al. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Nat Acad Sci U S A*. 1994;91:4892–4896.
15. Strachan FE, Spratt JC, Wilkinson IB, et al. Systemic blockade of the endothelin-B receptor increases peripheral resistance in healthy men. *Hypertension*. 1999;33:581–585.
16. Newby DE, Jalan R, Masumori S, et al. Peripheral vascular tone in patients with cirrhosis: role of the renin-angiotensin and sympathetic nervous systems. *Cardiovasc Res*. 1998;38:221–228.
17. Haber E, Koerner T, Page LB, et al. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocrinol Metab*. 1969;29: 1349–1355.
18. Kubasik N, Warren K, Sine HE. Evaluation of a new commercial radioassay kit for aldosterone using an iodinated tracer. *Clin Biochem*. 1979;12:59–61.
19. Wiinberg N, Walter-Larson S, Eriksen C, et al. An evaluation of semi-automatic blood pressure manometers against intra-arterial blood pressure. *J Ambulat Monit*. 1998;1:303–309.
20. Thomas SHL. Impedance cardiography using the Sramek-Bernstein method: accuracy and reproducibility at rest and during exercise. *Br J Clin Pharmacol*. 1992;34:467–476.
21. Levinsky NG, Levy M. Clearance techniques. In: Geiger SR, ed. *Handbook of Physiology, Section 8: Renal Physiology*. Baltimore, Md: Williams & Wilkins; 1973:103–117.
22. Honing ML, Hijmering ML, Ballard DE, et al. Selective ET(A) receptor antagonism with ABT-627 attenuates all renal effects of endothelin in humans. *J Am Soc Nephrol*. 2000;11:1498–1504.
23. Honing MLH, Bouter PK, Ballard DE, et al. ABT-627, a selective ETA-receptor antagonist, reduces proteinuria in patients with diabetes mellitus. In: *Regulation of Vascular Tone in Humans by Endothelium-Derived Mediators* [thesis]. Utrecht, Netherlands: Elinkwijk BV; 2000: 89–102.
24. Verhaar MC, Strachan FE, Newby DE, et al. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation*. 1998;97:752–756.
25. Love MP, Ferro CJ, Haynes WG, et al. Endothelin receptor antagonism in patients with chronic heart failure. *Cardiovasc Res*. 2000;47:166–172.
26. Goddard J, Webb DJ. Endothelin receptor antagonists: promising new agents in the management of cardiovascular disorders. *Drugs R & D*. 1999;2:1–12.
27. d'Uscio LV, Barton M, Shaw S, et al. Chronic ET(A) receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. *Cardiovasc Res*. 2002;53:487–495.
28. Tostes RC, Touyz RM, He G, et al. Endothelin A receptor blockade decreases expression of growth factors and collagen and improves matrix metalloproteinase-2 activity in kidneys from stroke-prone spontaneously hypertensive rats. *J Cardiovasc Pharmacol*. 2002;39:892–900.
29. Li JS, Turgeon A, Schiffrin EL. Effect of chronic treatment with two different ET(A) selective endothelin receptor antagonists on blood pressure and small artery structure of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. *Am J Hypertens*. 1998;11:554–562.

J Am Soc Nephrol 15: 2601–2610, 2004

Endothelin A Receptor Antagonism and Angiotensin-Converting Enzyme Inhibition Are Synergistic *via* an Endothelin B Receptor–Mediated and Nitric Oxide–Dependent Mechanism

JANE GODDARD,* CORINE ECKHART,* NEIL R. JOHNSTON,* ALLAN D. CUMMING,* ANDREW J. RANKIN,† and DAVID J. WEBB*

**Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom; and †Pfizer Global Research & Development, Sandwich, United Kingdom*

Abstract. Animal studies suggest that endothelin A (ETA) receptor antagonism and angiotensin-converting enzyme (ACE) inhibition may be synergistic. This interaction and the role of ETB receptors and endothelial mediators were investigated in terms of systemic and renal effects in humans in two studies. In one study, six subjects received placebo, the ETA receptor antagonist BQ-123 alone, and BQ-123 in combination with the ETB receptor antagonist BQ-788 after pretreatment with the ACE inhibitor enalapril (E) or placebo. In the other, six subjects who were pretreated with E received placebo, BQ-123, and BQ-123 with concomitant inhibition of nitric oxide (NO) synthase or cyclo-oxygenase (COX). Both were randomized, double-blind, crossover studies. Mean arterial pressure was reduced by BQ-123, an effect that was doubled during ACE inhibition (mean area under curve ± SEM; BQ-123, −2.3 ± 1.8%; BQ-123+E, −5.1 ± 1.1%; $P < 0.05$ *versus* placebo). BQ-123 increased effective renal blood flow (BQ-123, −0.1 ± 2.4%; BQ-123+E, 10.9 ± 4.2%; $P < 0.01$ *versus* BQ-123), reduced effective renal vascular resistance (BQ-123, −1.2 ± 3.1%; BQ-123+E, −12.8 ± 3.0%; $P < 0.01$ *versus* placebo and *versus* BQ-123), and increased urinary sodium excretion markedly (BQ-123, 2.6 ± 12.8%; BQ-123+E, 25.2 ± 12.6%; $P < 0.05$ *versus* BQ-123, $P < 0.01$ *versus* placebo and *versus* E) only during ACE inhibition. These effects were abolished by both ETB receptor blockade and NO synthase inhibition, whereas COX inhibition had no effect. In conclusion, the combination of ETA receptor antagonism and ACE inhibition is synergistic *via* an ETB receptor–mediated, NO-dependent, COX-independent mechanism. The reduction of BP and renal vascular resistance and associated substantial natriuresis make this a potentially attractive therapeutic combination in renal disease.

Endothelin-1 (ET-1) is a vasoactive peptide that is produced by the vascular endothelium (1) and acts through two receptors to regulate vascular tone. Vascular smooth muscle ETA and ETB receptors (2,3) mediate vasoconstriction, whereas endothelial ETB receptors mediate vasodilation through generation of nitric oxide (NO) and prostanoids (3). Angiotensin II (Ang II) is another powerful vasoconstrictor involved in the regulation of vascular tone, and there is considerable evidence for an interaction between the endothelin and renin-angiotensin systems (4). Ang II increases ET-1 transcription and secretion *in vitro* in a variety of cell types, including endothelial and vascular smooth muscle cells (5,6), and ET receptor antagonists attenuate the acute hemodynamic effects of Ang II in rats *in vivo* (7,8). However, this is by no means a uniform finding (9) and has not been replicated in dogs (10) or humans (11). Data in animals suggest that concomitant ET blockade and angiotensin-converting enzyme (ACE) inhibition produce changes greater than those seen with blockade of either system alone (12–15). In addition, clinical studies that demonstrated major hemodynamic effects of ET receptor antagonists have generally been performed in patients who were already receiving ACE inhibitors (16,17).

ACE inhibition, by promoting the effects of bradykinin (18), and ET-1 acting on endothelial ETB receptors (19) both enhance endothelium-dependent vasodilation. Vascular studies in humans suggest that the vasodilator effects of ETA receptor antagonism are dependent on the unblocked ETB receptor and NO generation (20). The aim of this study, therefore, was to explore the possible systemic and renal interaction between ET receptor antagonism and ACE inhibition and the mechanisms by which such an interaction might occur. We hypothesized that previous ACE inhibition would augment the systemic and renal hemodynamic response to ETA receptor antagonism and that this interaction would be dependent on NO production and mediated, at least in part, through the ETB receptor.

Received April 25, 2004. Accepted July 12, 2004.
Correspondence to Prof. David J. Webb, Clinical Research Centre, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU, UK. Phone: +44-131-537-2006; Fax: +44-0870-134-0897; E-mail: d.j.webb@ed.ac.uk
1046-6673/1510-2601
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000141313.84470.4B

Materials and Methods

A total of eight healthy men (six in each protocol) were recruited to the studies, which were performed in the University of Edinburgh's Clinical Research Centre with the approval of the local research ethics committee and the written informed consent of each participant. The investigations conformed to the principles outlined in the Declaration of Helsinki.

For 3 d before each study, participants adhered to a standard diet that contained 150 mmol of sodium. All participants abstained from over-the-counter medication for 2 wk, alcohol, nicotine, and caffeine-containing products for 24 h and had a light breakfast before attending on each study day. All studies were carried out in a quiet, temperature-controlled room, at 22 to 24°C, with the participant recumbent throughout, except when voiding urine.

Drugs

BQ-123 (Clinalfa AG, Laufelfingen, Switzerland), a selective ETA receptor antagonist (21), was infused at 100 and 1000 nmol/min for 15 min at each dose. These doses were selected from a previous study as having a threshold and maximum hemodynamic effect in healthy control subjects (22). The higher dose produces a peak plasma concentration of BQ-123 (22) 7-fold higher than the K_i for ETA receptor inhibition and 20-fold lower than the K_i for ETB receptor inhibition (21), confirming ETA selectivity, and did not increase plasma ET-1, an index of ETB receptor blockade (23). BQ-788 (Clinalfa AG), a selective ETB receptor antagonist (24), was infused at 30 and 300 nmol/min for 15 min, doses shown to be hemodynamically active in a previous systemic dose-ranging study (25). Drugs were dissolved in physiologic saline (0.9%; Baxter Healthcare Ltd, Thetford, UK) and infused intravenously at a constant rate of 1 ml/min *via* an 18 standard wire gauge (SWG) cannula sited in the right antecubital fossa. Saline was administered as placebo.

Enalapril (Dexcel Pharma Ltd, Daventry, UK) was the chosen ACE inhibitor and was administered orally for 5 d before each visit at a dose of 20 mg twice daily, taking the final dose at 8:30 a.m. on each study day. This dose was chosen from data suggesting that it would achieve significant inhibition of serum ACE activity (26,27), with steady-state plasma concentrations reached by 3 to 4 d (28). With this dosing regimen, the maximal effect of enalapril occurs 2 to 3 h after administration and persists for 5 to 7 h (27,29). By administering a final dose 2.5 h before baseline measurements, therefore, we aimed to achieve maximal ACE inhibition throughout each study visit.

The nonselective cyclo-oxygenase (COX) inhibitor indomethacin was administered as a single 100-mg oral dose at 8:30 a.m. on the relevant study day to reach peak plasma concentrations by the time of baseline measurements. The dose is known to inhibit prostaglandin production over the time course of the study, as evidenced by decreased urinary excretion rates of prostaglandins (30). N^G-monomethyl-L-arginine (L-NMMA; Clinalfa AG, Laufelfingen, Switzerland) was administered at a dose of 3 mg/kg over 5 min as an NO synthase inhibitor, with each dose of BQ-123, during the relevant study day. This dose is sufficient to cause a small transient increase in BP (by ~7 to 10%) and decrease renal blood flow (by ~10%) (31). Thereafter, BP returns to baseline by 30 min and renal blood flow by 60 min, with a plasma half-life of 75 min (31).

Para-aminohippurate sodium (PAH; Clinalfa AG) and inutest (Fresenius Pharma, Austria) were dissolved in dextrose 5% (Baxter Healthcare) and administered as a bolus loading dose of 0.4 g of PAH and 3.5 g of inutest in 100 ml of dextrose over 15 min and a maintenance infusion of 6.6 g/L PAH and 10 g/L inutest at a rate of 2 ml/min *via* an 18 SWG cannula in the left antecubital fossa. All drugs were prepared from sterile stock solutions on the day of the study.

Assays

At prespecified time points, venous blood was collected into tubes that contained EDTA (Sarstedt, Newton, CA) for the measurement of PAH, inulin, and hematocrit (Hct) and into plain tubes (Sarstedt) for the measurement of plasma sodium. Twenty-milliliter aliquots of urine from each voiding were collected into plain tubes for the measurement of urinary PAH, inulin, and sodium.

Hct was measured on whole blood using a Coulter counter. All other blood samples were centrifuged immediately at 1000 × *g* at 4°C for 20 min, and plasma and urine were stored in plain tubes at −80°C. Inulin concentrations were determined by spectrophotometry after hydrolysis to fructose, and PAH and BQ-123 (22) were determined by HPLC. BQ-788 assay was not sufficiently sensitive for its detection in plasma. Urinary and plasma sodium concentrations were determined using standard flame photometry. Subaliquots of plasma were used to measure serum ACE activity by generation of Ang II from Ang I (32).

Study Design

Both studies were double blind and placebo controlled. Visits were separated by at least 1 wk. The first study was designed to examine the interaction between ET receptor antagonism and ACE inhibition, and the second was designed to examine the effect of NO synthase inhibition and inhibition of prostaglandin synthesis on this interaction.

Study 1. Participants attended a total of six visits. On two sets of three visits, they received placebo, BQ-123, or the combination of BQ-123 and BQ-788 in a randomized order. With one set, they received pretreatment with enalapril and the other placebo.

Study 2. Participants attended a total of four visits. On each visit, they received pretreatment with enalapril. During the study day, they then received placebo, BQ-123, BQ-123 + indomethacin, or BQ-123 + L-NMMA in a randomized order.

Study Protocol

On each study day, 18-SWG cannulae were sited in an antecubital vein in each arm. At 8:30 a.m., diuresis was induced by the administration of 500 ml of 5% dextrose over 30 min through the cannula in the left arm. The loading dose of PAH and inutest was administered through the same cannula from 8:45 a.m.. Thereafter, maintenance infusions of PAH and inutest, and 5% dextrose at 260 ml/h, continued throughout the study. BP, cardiac output, and heart rate were recorded using well-validated noninvasive automated techniques every 15 min (33,34), and urine was collected every 30 min by spontaneous voiding. After a 2-h equilibration period, baseline measurements were made over two 30-min urine collection periods. The lower dose of ET receptor antagonist was then administered at 12:00 p.m. through the right antecubital cannula, followed by three 30-min collection periods. At 1:30 p.m., the higher dose of antagonist was administered followed by five additional 30-min collection periods.

At the midpoint of each urine collection period, blood was sampled from the right antecubital cannula for the measurement of PAH, inulin, sodium, and Hct. BQ-123 was measured before and at 15, 45, and 90 min after the start of each dose of antagonist and at the end of the study, and serum ACE activity was determined at 8:30 a.m. and at 2:30 p.m. (60 min after the start of the higher dose of antagonist).

Statistical Analyses

Data were stored and analyzed using a Microsoft Excel data analysis package (Excel 5.0, Microsoft, Wokingham, UK). Demographic

data are expressed as mean ± SEM. BP at each time point was calculated as the mean of two recordings and represented as mean arterial pressure (MAP; = diastolic BP + 1/3 pulse pressure). Bioimpedance data at each time point were calculated as the mean of four recordings, each the average of 15 consecutive heart beats. Data were corrected using body surface area to give cardiac index for direct comparison of participants. Systemic vascular resistance index (SVRI) was calculated by dividing MAP by cardiac index and expressed in dyne.s m^2/cm^5. GFR and effective renal plasma flow (ERPF) were calculated from inulin and PAH clearances, respectively (35). Effective renal blood flow (ERBF) was calculated by dividing ERPF by (1 − Hct); effective renal vascular resistance (ERVR) was calculated by dividing MAP by ERBF. Effective filtration fraction (EFF) was calculated as GFR divided by ERPF × 100%. Urinary sodium excretion rate was calculated as urinary sodium × urinary flow rate; and fractional excretion of sodium (FeNa) was calculated as urinary sodium × plasma inulin, divided by plasma sodium × urine inulin.

Study baseline data were calculated as the mean of the two time points immediately preceding the administration of the first study drug. Hemodynamic results are expressed as maximum placebo-corrected change from baseline (mean ± SEM). Statistical analysis was performed on untransformed data, and responses were examined by repeated measures ANOVA. In addition, area under the curve was calculated as a summary statistic of each time curve, and responses were compared by paired *t* test. Statistical significance was taken at the 5% level. Power calculations from previous studies (22,36) suggested that, with $n = 6$ participants, the studies had 80% power to achieve statistical significance for systemic hemodynamic indices. In study 1, the response to BQ-123 and BQ-123/788 in the presence or absence of enalapril was preidentified as the comparison of interest. In study 2, comparison of BQ-123+enalapril with the other three drug combinations individually was preidentified as the comparison of interest.

Results

Six participants were recruited to study 1, and seven were recruited to study 2. Participants completed all phases of study 1 without adverse event. During study 2, one participant experienced an increase in MAP of 50 mmHg after indomethacin administration and before receiving BQ-123 and was withdrawn from the study. Baseline characteristics for the eight participants who completed the studies (four of whom were common to both studies) are shown in Table 1.

Table 1. Participant demographic data[a]

Age (yr)	47 ± 5	(23–64)
BMI (kg/m^2)	25 ± 2	(18–31)
Mean arterial pressure (mmHg)	88.5 ± 2.7	(71.4–99.4)
Creatinine (mg/dl)[b]	1.01 ± 0.08	(0.70–1.35)
Creatinine clearance (ml/min)	96 ± 7	(70–132)
24-h urinary sodium excretion (mEq/24 h)	118 ± 15	(64–185)
Cholesterol (mg/dl)[c]	201 ± 12	(151–236)

[a] Values are given as mean ± SEM with the range of values given in parentheses.
[b] To convert to μmol/L, multiply by 88.4.
[c] To convert to mmol/L, multiply by 0.0259.

Study 1: Interaction between ET Receptor Antagonism and ACE Inhibition

Systemic and Renal Hemodynamics. Administration of placebo, either alone or after pretreatment with enalapril, did not alter systemic hemodynamics. MAP was reduced after both BQ-123 by 4.2 ± 1.6 mmHg (ANOVA $P < 0.05$ *versus* placebo) and BQ-123/788 by 4.4 ± 2.0 mmHg (Figure 1A). After pretreatment with enalapril, the BP reduction after BQ-123 was almost doubled, with MAP falling by 8.3 ± 3.0 mmHg ($P < 0.01$ *versus* BQ-123 alone). However, this synergy was not seen when BQ-788 was co-administered with BQ-123, when MAP was reduced by 4.3 ± 3.3 mmHg. SVRI followed a similar pattern (Figure 1B).

Placebo, enalapril alone, BQ-123, and BQ-123/788 all were neutral in respect of ERBF, ERVR, EFF, and GFR. After pretreatment with enalapril, BQ-123 increased ERBF by 146 ± 41 ml/min ($P < 0.01$ *versus* placebo and *versus* BQ-123 alone), reduced ERVR by 32 ± 15 mmHg/min per L ($P < 0.01$ *versus* placebo and *versus* BQ-123 alone), and reduced EFF by 3.4 ± 2.5% ($P < 0.05$ *versus* placebo, $P < 0.01$ *versus* BQ-123 alone; Figure 2). By contrast, after pretreatment with enalapril, BQ-123/788 reduced ERBF by 224 ± 31 ml/min ($P < 0.01$ *versus* placebo and *versus* BQ-123/788 alone) and increased ERVR by 26 ± 6 mmHg/min per L ($P < 0.05$ *versus* BQ-123/788 alone).

Sodium Excretion. No significant natriuresis was observed after placebo, enalapril alone, and either BQ-123 or BQ-123/788 alone. However, after pretreatment with enalapril, BQ-123 produced a striking increase in urinary sodium excretion with a maximum excretion rate of 58 ± 27 μmol/min ($P < 0.01$ *versus* placebo and BQ-123 alone). FeNa followed a similar pattern. As with renal hemodynamics, this increase was not observed with BQ-123/788 (Figure 3).

Serum ACE Activity. Compared with placebo, pretreatment with enalapril reduced serum ACE activity by 75% at baseline (36.4 ± 4.3 *versus* 9.1 ± 2.3 U; $P < 0.01$) and by 79% at 150 min (26.5 ± 2.1 *versus* 5.4 ± 1.7 U; $P < 0.01$). ET receptor antagonist administration did not alter ACE activity.

Plasma BQ-123. BQ-123 was detectable in plasma at 15 min after the start of the low-dose and at 15 and 45 min after the start of the high-dose infusion. Peak BQ-123 concentrations tended to be higher after pretreatment with enalapril, although the difference was not statistically significant (BQ-123, 1.26 ± 0.18 pg/ml; BQ-123/788, 1.32 ± 0.23; BQ-123 + enalapril, 2.06 ± 0.65; BQ-123/788 + enalapril, 2.32 ± 0.53).

Study 2: Effect of NO Synthase Inhibition and Inhibition of Prostaglandin Synthesis on Combined ETA Receptor Antagonism and ACE Inhibition

Systemic and Renal Hemodynamics. MAP was reduced by 6.0 ± 1.7 mmHg after BQ-123 in the presence of enalapril. This effect was augmented by indomethacin, with MAP falling by 11.0 ± 2.3 mmHg at the end of the study ($P < 0.01$ *versus* BQ-123; Figure 4) and abolished by L-NMMA ($P < 0.01$

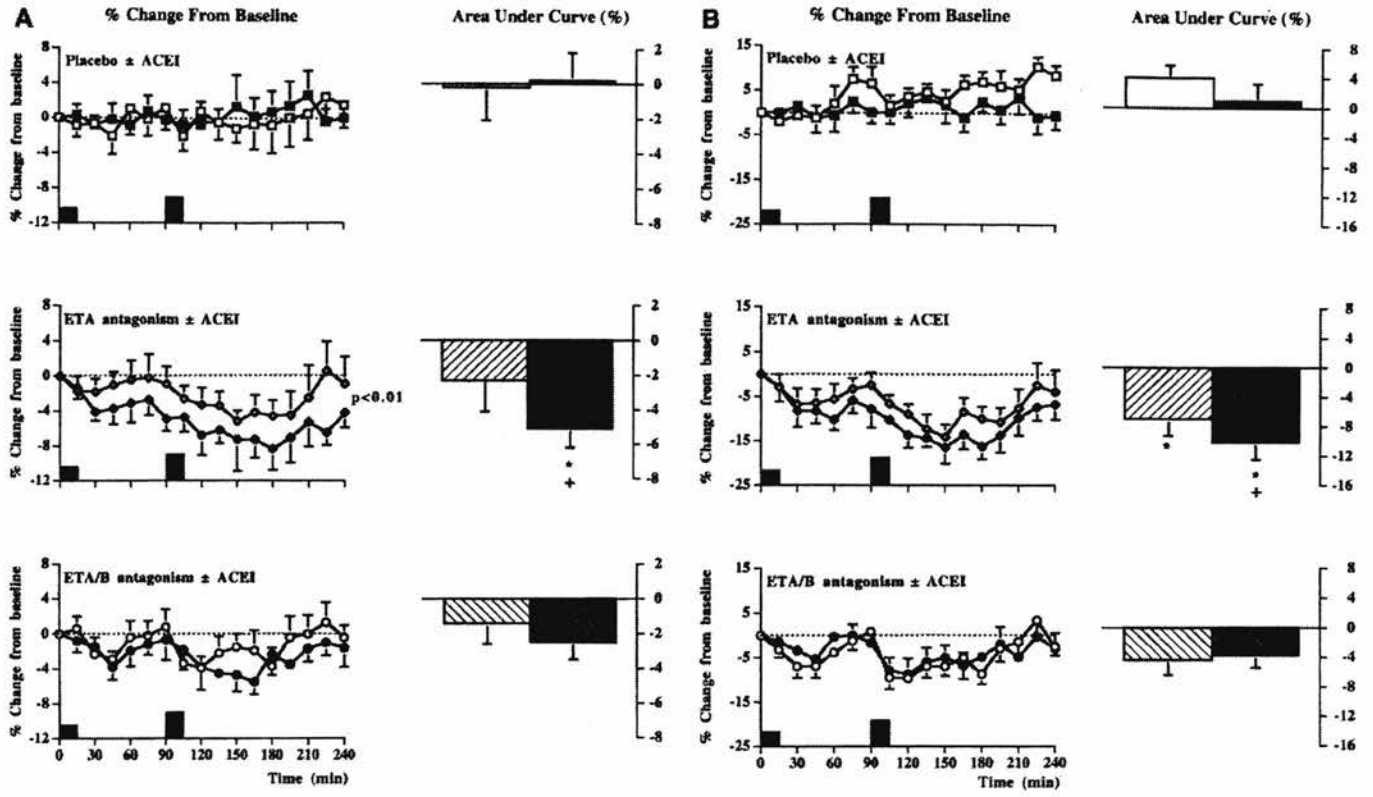


Figure 1. (A) Effects of endothelin (ET) receptor antagonism and angiotensin-converting enzyme (ACE) inhibition on mean arterial pressure. (Left) Mean of percentage change from baseline ± SEM; □, placebo; ■, placebo + enalapril; ◇, BQ-123; ◆, BQ-123 + enalapril; ○, BQ-123/788; ●, BQ-123/788 + enalapril; ▮, administration of study drug. (Right) Mean area under curve of percentage change from baseline ± SEM; □, placebo; ■, placebo + enalapril; ▨, BQ-123; ▨, BQ-123 + enalapril; ▧, BQ-123/788; ▧, BQ-123/788 + enalapril. $^{*}P < 0.05$ *versus* placebo, $^{+}P < 0.05$ *versus* placebo + enalapril, §$P < 0.05$ *versus* BQ-123. (B) Effects of ET receptor antagonism and ACE inhibition systemic vascular resistance index. Legend as for A.

versus BQ-123). Again, SVRI followed a similar pattern (Figure 4). Indomethacin did not affect the renovascular changes induced by BQ-123 + enalapril, but L-NMMA abolished the effects of BQ-123 + enalapril on both ERBF and ERVR ($P < 0.01$) (Figure 4).

Sodium Excretion. The natriuresis produced by the combination of BQ-123 and enalapril was attenuated by indomethacin ($P < 0.01$ *versus* BQ-123 + enalapril) and abolished by L-NMMA ($P < 0.01$ *versus* BQ-123 + enalapril) (Figure 4).

Discussion

In these studies, we demonstrated a synergy between the effects of ACE inhibition and ETA receptor antagonism in humans, affecting both systemic and renal hemodynamics, as well as renal tubular function. This synergy is abolished by either ETB receptor blockade or NO synthase inhibition but not COX inhibition. We conclude that ETA receptor antagonism and ACE inhibition act synergistically through an ETB receptor–mediated, NO-dependent, and COX-independent mechanism.

The ET and renin-angiotensin systems are known to interact (4), and an *in vivo* synergistic effect between ETA receptor antagonism and ACE inhibition has been demonstrated in animals (12) and between ET receptor antagonism and angiotensin AT1 receptor antagonism in humans (37). We have shown, in healthy men who were subjected to systemic ACE inhibition with a clinically relevant dose of enalapril, that the effects of ETA receptor blockade on systemic hemodynamics are enhanced. With respect to BP, ACE inhibition almost doubled the hypotensive effect of ETA receptor blockade. In addition, in contrast to the absence of an effect of ETA receptor antagonism alone on the renal circulation, the combination with ACE inhibition increased renal perfusion, in association with a fall in EFF. Thus, by inference, this combination causes preferential efferent renal arteriolar vasodilation that should be associated with a fall in glomerular capillary pressure. Such changes in glomerular hemodynamics have the potential to be renoprotective in a manner analogous to the effect of ACE inhibition alone. In addition, this combination produced a striking natriuresis that was still developing to the end of the study, 4 h after initial administration of BQ-123. At its maximum, this natriuresis measured 10 mmol/h, a clinically important degree of sodium loss.

Our results also demonstrate that although maximal hemodynamic changes occurred after the higher dose of BQ-123, potentially useful systemic and renal hemodynamic changes were achieved after a lower dose of BQ-123 in the presence of ACE inhibition. As adverse effects in clinical trials with ET receptor antagonists seem to be largely dose related (38), this

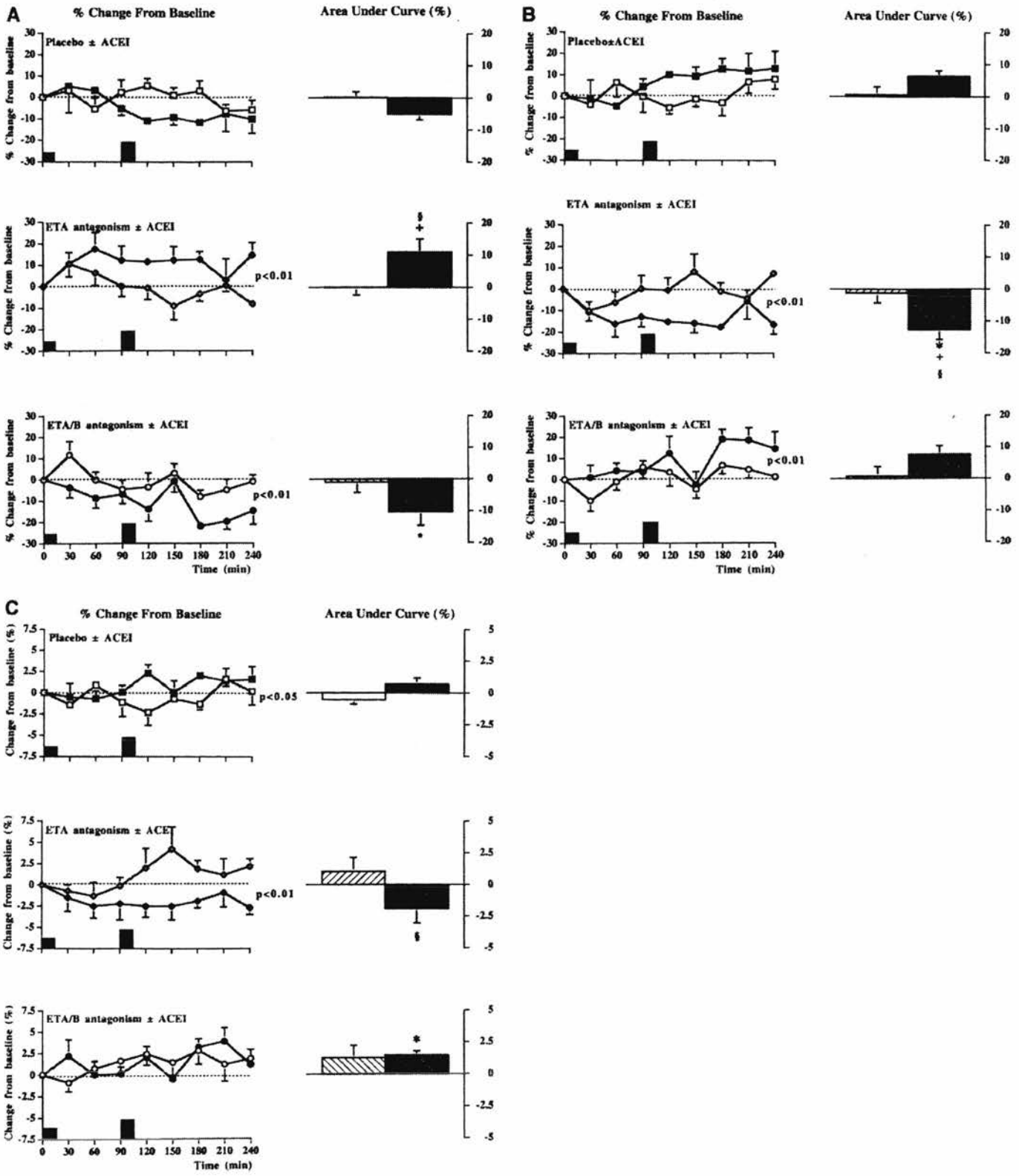


Figure 2. (A) Effects of ET receptor antagonism and ACE inhibition on effective renal blood flow. Legend as for Figure 1A. (B) Effects of ET receptor antagonism and ACE inhibition on effective renal vascular resistance. Legend as for Figure 1A. (C) Effects of ET receptor antagonism and ACE inhibition on effective filtration fraction. Legend as for Figure 1A.

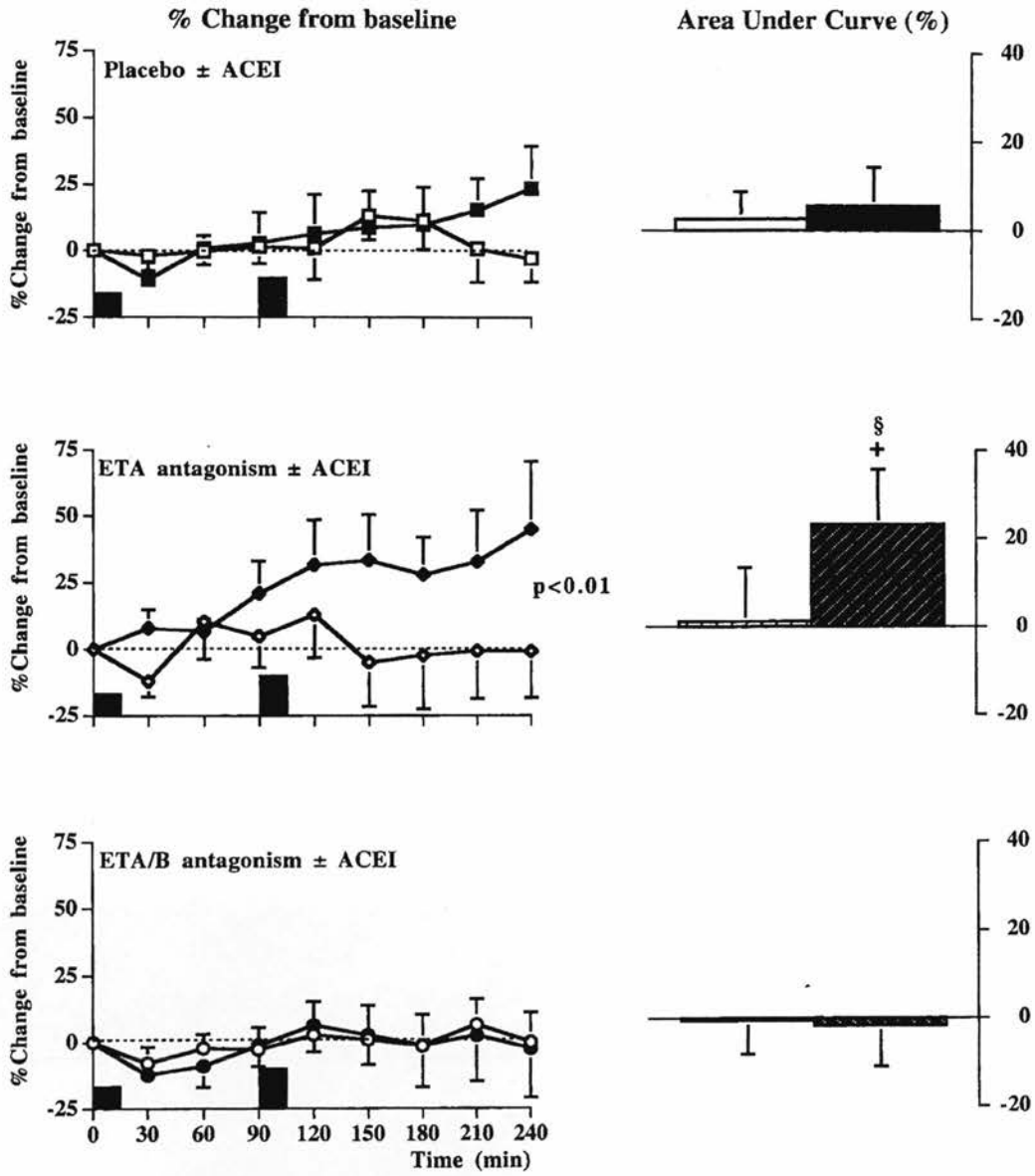


Figure 3. Effects of ET receptor antagonism and ACE inhibition on fractional urinary excretion of sodium. Legend as for Figure 1A.

combination might allow the use of a lower and better tolerated dose of ETA receptor antagonist without compromising efficacy.

Montanari *et al.* (37) demonstrated that ETA receptor antagonism can produce renal hemodynamic changes under conditions of angiotensin AT1 receptor blockade and that this action is inhibited by NO synthase inhibition. We demonstrated a similar synergy between ETA receptor antagonism and ACE inhibition, again dependent on NO, but extended this work by showing that this synergistic effect is abolished, for all indices studied, when the ETB receptor is blocked, suggesting that the ETB receptor is crucial to the mechanism of this interaction. As both L-NMMA and BQ-788 are vasoconstrictors, it is possible that their ability to abolish the vasodilator effect of enalapril and BQ-123 is nonspecific. However, indomethacin has also been shown to produce renal vasoconstriction in healthy subjects (39) but had no effect on the renal vasodilation seen in this study after enalapril and BQ-123. Similarly, previous studies in healthy volunteers have demonstrated that although this dose of BQ-788 alone produces systemic and renal vasoconstriction, it does not abolish the vasodilator effects of BQ-123 (36), suggesting that the effect seen here is specific to the interaction between ACE inhibition and ETA receptor antagonism. In an experimental rat model of interstitial renal fibrosis, enalapril treatment was shown to increase ETB mRNA expression (40), providing a possible mechanism for this ET-1/ACE interaction. It is possible that ETA receptor antagonism then results in displacement of endogenous ET-1 from the ETA receptor onto the unblocked, upregulated ETB receptor.

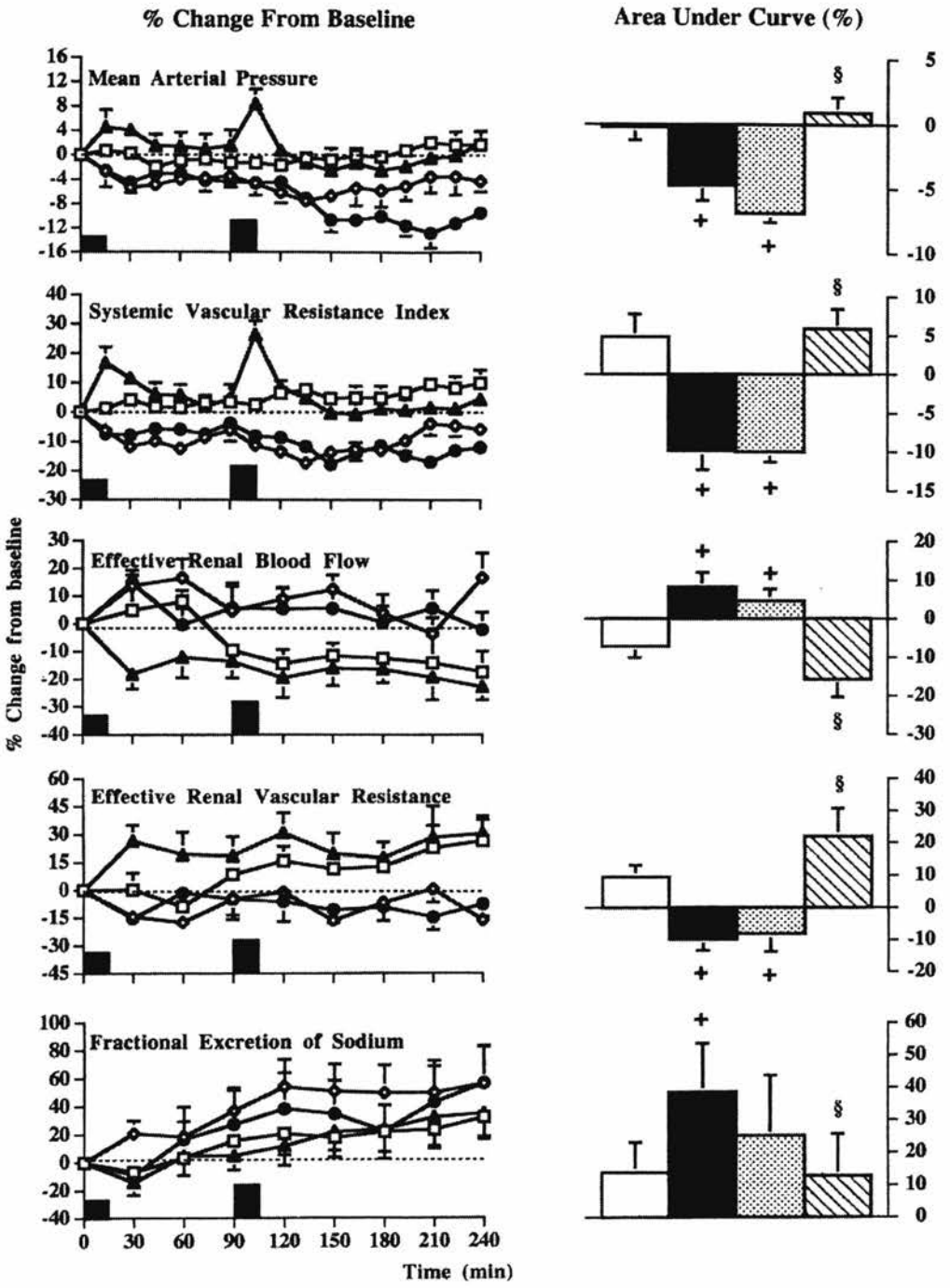


Figure 4. Systemic and renal hemodynamic and tubular effects of N^G-monomethyl-L-arginine (L-NMMA) and indomethacin on the response to the combination of BQ-123 and enalapril. (Left) Mean of percentage change from baseline ± SEM, except for effective filtration fraction (EFF), which was mean change from baseline (%); □, placebo + enalapril; ◇, BQ-123 + enalapril; ●, BQ-123 + enalapril + indomethacin; ▲, BQ-123 + enalapril + L-NMMA; ▮, administration of study drug. (Right) Mean area under curve of percentage change from baseline ± SEM (EFF, mean change from baseline [%]). □, placebo + enalapril; ■, BQ-123 + enalapril; ▨, BQ-123 + enalapril + indomethacin; ▧, BQ-123 + enalapril + L-NMMA. $^+P < 0.05$ *versus* placebo + enalapril, §$P < 0.05$ *versus* BQ-123 + enalapril.

These studies were performed in subjects in a salt-replete state (mean 24-h urine excretion, 119 ± 6 mmol). A recent study suggested that ET-1 plays a role in angiotensin-dependent hypertension in humans (41), particularly with respect to BP and proteinuria. Salt depletion, with enhancement of renin-angiotensin activity, therefore might further enhance the synergy seen between ETA receptor antagonism and ACE inhibition.

It is interesting that in the presence of ACE inhibition in our study, combined ETA/B receptor antagonism tended to increase renal vascular tone and reduce blood flow, underlining the importance of ETB-mediated vasodilation. This finding has important implications for the therapeutic use of ET receptor antagonists, suggesting that, in conjunction with ACE inhibitors, ETA receptor antagonists may be superior to nonselective ETA/B receptor antagonists with respect to some important hemodynamic effects. Indeed, we previously demonstrated in patients who had chronic renal failure and were already being treated with ACE inhibitors that ETA but not combined ETA/B receptor antagonism increases renal blood flow (36). The current demonstration of an ETB receptor–dependent, synergistic interaction between ETA receptor antagonism and ACE inhibition suggests that these differences observed in renal patients may, at least in part, be due to concomitant chronic treatment with ACE inhibitors.

Although prostacyclin is the major COX product of macrovascular endothelium in vitro, COX activity can produce both vasoconstrictor and vasodilator achidonic acid derivatives. Prostaglandins stimulate renin release (42). Thus, indomethacin may produce both vasodilation, by inhibition of renin-mediated Ang II generation and COX-1–mediated thromboxane A2 synthesis, and vasoconstriction, by blocking synthesis of vasodilator prostanoids. Studies in animals also suggest that vasoconstrictor COX products, such as thromboxane A2 and prostaglandin H2, might be implicated in ET-1–induced vasoconstriction, particularly in disease models (43–47). The greater fall in BP when indomethacin was co-administered with enalapril and BQ-123 may represent blockade of the action of these constrictor COX products. There is evidence from studies in animals that vasodilator prostaglandins may additionally be involved in the actions of ET-1 in the renal circulation, particularly the renal medulla (45,48). We could not demonstrate, however, any inhibitory effect of indomethacin on either the systemic or the renal hemodynamic effects of the combination of ACE inhibition and ETA receptor antagonism. With respect to renal blood flow, however, clearance studies only measure total renal blood flow. It is possible, therefore, that opposite changes are occurring in the renal cortex and medulla.

With respect to natriuresis, knockout and antagonist studies in animals have implicated ETB receptors, linked to NO production, in ET-1–mediated sodium excretion (49). COX inhibition, however, augments big ET-1–mediated natriuresis, suggesting an antinatriuretic role for prostaglandins (50). Our studies demonstrate, in the presence of ACE inhibition, an ETB-dependent natriuresis that is mediated by both NO and, to a lesser extent, prostanoids.

In summary, in this mechanistic study, we demonstrated that ACE inhibition increases the systemic hemodynamic effects of ETA receptor antagonism, at both low and high dose, and unmasks a renal hemodynamic and tubular effect. This synergy is mediated mainly by NO and requires an unblocked ETB receptor. These findings would preferentially support the further investigation of selective ETA receptor antagonism, over combined ETA/B receptor antagonism, as a useful adjunct to ACE inhibition in the management of the systemic and renal vascular consequences of diseases characterized by vasoconstriction. This may be particularly important in circumstances in which sodium loss would also be beneficial, such as hypertension and chronic renal failure. This interaction should now be explored in longer term studies in patients with such conditions.

Acknowledgments

D.J.W. was the recipient of a Research Leave Fellowship from the Wellcome Trust (no. WT0526330). J.G. was supported by an educational grant from Pfizer Global Research & Development (Sandwich, UK).

Part of this work has previously appeared in abstract form: Goddard J, Eckhart C, Cumming AD, Rankin AJ, Webb AJ: Endothelin-A receptor antagonism is synergistic with ACE inhibition via an ETB receptor mediated nitric oxide dependent mechanism. British Pharmacologic Society 2001 [Abstract]. *Br J Clin Pharmacol* 53: 428P, 2002.

We thank Ms. F. Howe and Ms. L. Coppard for excellent technical assistance in this project and Prof. T.J. Rabelink for help with data interpretation.

References

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332: 411–415, 1988
2. Moreland S, McMullen DM, Delaney CL, Lee VG, Hunt JT: Venous smooth muscle contains vasoconstrictor ETB-like receptors. *Biochem Biophys Res Commun* 184: 100–106, 1992
3. Gray G, Webb DJ: The endothelin system and its potential as a therapeutic target in cardiovascular disease. *Pharmacol Ther* 72: 109–148, 1996
4. Rossi GP, Sacchetto A, Cesari M, Pessina AC: Interactions between the endothelin-1 and the renin-angiotensin-aldosterone system. *Cardiovasc Res* 43: 300–207, 1999
5. Imai T, Hirata Y, Emori T, Yanagisawa M, Masaki T, Marumo F: Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. *Hypertension* 19: 753–757, 1992
6. Kohno M, Horio T, Ikeda M, Yokokawa K, Fuki T, Yasunari K, Kurihara N, Takeda T: Angiotensin II stimulates endothelin-1 secretion in cultured rat mesangial cells. *Kidney Int* 42: 860–866, 1992
7. Balakrishnan SM, Wang HD, Gopalakrishnan V, Wilson TW, McNeill JR: Effect of an endothelin antagonist on hemodynamic responses to angiotensin II. *Hypertension* 28: 806–809, 1996
8. Riggleman A, Harvey J, Baylis C: Endothelin mediates some of the renal actions of acutely administered angiotensin II. *Hypertension* 38: 105–109, 2001
9. Gardiner SM, March JE, Kemp PA, Bennett T: Cardiovascular responses to angiotensins I and II in normotensive and hypertensive rats; effects of NO synthase inhibition or ET receptor antagonism. *Br J Pharmacol* 128: 1795–1803, 1999
10. Boemke W, Hocher B, Schleyer N, Krebs MO, Kaczmarczyk G: Hemodynamic, renal, and endocrine responses to acute ETA blockade at different ANG II plasma levels. *Am J Physiol* 280: R1322–R1331, 2001
11. Bayerle-Eder M, Langenberger H, Pleiner J, Polska E, Mensik C, Eichler HG, Wolzt M, Schmetterer L: Endothelin ETA receptor-subtype specific antagonism does not mitigate the acute systemic

or renal effects of exogenous angiotensin II in humans. *Eur J Clin Invest* 32: 230–235, 2002
12. Berthold H, Munter K, Just A, Kirchheim HR, Ehmke H: Contribution of endothelin to renal vascular tone and autoregulation in the conscious dog. *Am J Physiol* 279: F417–F424, 1999
13. Amann K, Simonaviciene A, Medwedewa T, Koch A, Orth S, Gross ML, Haas C, Kuhlmann A, Linz W, Scholkens B, Ritz E: Blood pressure-independent additive effects of pharmacologic blockade of the renin-angiotensin and endothelin systems on progression in a low-renin model of renal damage. *J Am Soc Nephrol* 12: 2572–2584, 2001
14. Bauersachs J, Fraccarollo D, Schafer A, Ertl G: Angiotensin-converting enzyme inhibition and endothelin antagonism for endothelial dysfunction in heart failure: Mono or combination therapy. *J Cardiovasc Pharmacol* 40: 594–600, 2002
15. Mulder P, Boujedaini H, Richard V, Henry JP, Renet S, Munter K, Thuillez C: Long-term survival and hemodynamics after endothelin-a receptor antagonism and angiotensin-converting enzyme inhibition in rats with chronic heart failure: Monotherapy versus combination therapy. *Circulation* 106: 1159–1164, 2002
16. Cowburn PJ, Cleland JGF, McArthur JD, MacLean MR, McMurray JJV, Dargie HJ: Short-term haemodynamic effects of BQ-123, a selective endothelin ETA-receptor antagonist, in chronic heart failure. *Lancet* 352: 201–202, 1998
17. Sutsch G, Kiowski W, Yan X-W, Hunziker P, Christen S, Strobel W, Kim J-H, Rickenbacher P, Bertel O: Short-term oral endothelin-receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. *Circulation* 98: 2262–2268, 1998
18. Benjamin N, Cockcroft JR, Collier JG, Dollery CT, Ritter JM, Webb DJ: Local inhibition of converting enzyme and vascular responses to angiotensin and bradykinin in the human forearm. *J Physiol* 412: 543–555, 1989
19. Hirokazu T, Ende H, Magazine I, Bahou WF, Goligorsky MS: Molecular and functional characterization of the non-isopeptide-selective ETB receptor in endothelial cells. *J Biol Chem* 269: 21778–21785, 1994
20. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ: Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation* 97: 752–756, 1998
21. Ihara M, Ishikawa K, Fukuroda T, Saeki T, Funabashi K, Fukami T, Suda H, Yano M: In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ETA receptor. *J Cardiovasc Pharmacol* 20[Suppl 12]: S11–S14, 1992
22. Spratt JCS, Goddard J, Patel N, Strachan FE, Rankin AJ, Webb DJ: Systemic ETA receptor antagonism with BQ-123 blocks ET-1 induced forearm vasoconstriction and decreases peripheral vascular resistance in healthy men. *Br J Pharmacol* 134: 648–654, 2001
23. Dupuis J, Stewart DJ, Cernacek P, Gosselin G: Human pulmonary circulation is an important site for both clearance and production of endothelin-1. *Circulation* 94: 1278–1284, 1996
24. Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami T, Ozaki S, Nagase T, Nishikibe M, Yano M: Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Natl Acad Sci U S A* 91: 4892–4896, 1994
25. Strachan FE, Spratt JC, Wilkinson IB, Johnston N, Gray GA, Webb DJ: Systemic blockade of the endothelin-B receptor increases peripheral vascular resistance in healthy men. *Hypertension* 33: 581–585, 1999
26. MacFadyen RJ, Elliot HL, Meredith PA, Reid JL: Haemodynamic and hormonal responses to oral enalapril in salt depleted normotensive man. *Br J Clin Pharmacol* 35: 299–301, 1993
27. Ribeiro W, Muscara MN, Martins AR, Moreno HJ, Mendes GB, de Nucci G: Bioequivalence study of two enalapril maleate tablet formulations in healthy male volunteers. *Br J Clin Pharmacol* 50: 399–405, 1996
28. Davies RO, Gomez HJ, Irvin JD, Walker JF: An overview of the clinical pharmacology of enalapril. *Br J Clin Pharmacol* 18[Suppl 2]:215S–229S, 1984
29. Brunner DB, Desponds G, Biollaz J, Keller I, Ferber F, Gavras H, Brunner HR, Schelling JL: Effects of a new angiotensin converting enzyme inhibitor MK 421 and its lysine analogue on the components of the renin system in healthy subjects. *Br J Clin Pharmacol* 11: 461–467, 1981
30. Olsen NV, Jensen NG, Hansen JM, Christensen NJ, Fogh-Andersen N, Kanstrup IL: Non-steroidal anti-inflammatory drugs and renal response to exercise: A comparison of indomethacin and nabumetone. *Clin Sci* 97: 457–465, 1999
31. Haynes WG, Hand MF, Dockrell MEC, Eadington DW, Lee MR, Hussein Z, Benjamin N, Webb DJ: Physiological role of nitric oxide in regulation of renal function in humans. *Am J Physiol* 272: F364–F371, 1997
32. Holmquist B, Bunning P, Riordan JF: A continuous spectrophotometric assay for angiotensin converting enzyme. *Annal Biochem* 95: 540–548, 1979
33. Thomas SHL: Impedance cardiography using the Sramek-Bernstein method: Accuracy and reproducibility at rest and during exercise. *Br J Clin Pharmacol* 34: 467–476, 1992
34. Wiinberg N, Walter-Larson S, Eriksen C, Nielsen PE: An evaluation of semi-automatic blood pressure manometers against intra-arterial blood pressure. *J Ambul Monit* 1: 303–309, 1998
35. Levinsky NG, Levy M: Clearance techniques. In: *Handbook of Physiology: Section 8: Renal Physiology*, edited by Orloff J, Berliner RW, Baltimore, Williams & Wilkins, 1973, pp 103–117
36. Goddard J, Johnston N, Hand MF, Cumming AD, Rabelink TJ, Rankin AJ, Webb DJ: Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: A comparison of selective and combined endothelin receptor blockade. *Circulation* 109: 1186–1193, 2004
37. Montanari A, Carra N, Perinotto P, Iori V, Fasoli E, Biggi A, Novarini A: Renal hemodynamic control by endothelin and nitric oxide under angiotensin II blockade in man. *Hypertension* 39: 715–720, 2002
38. Kalra PR, Moon JC, Coats AJ: Do results of the ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) study spell the end for non-selective endothelin antagonism in heart failure? *Int J Cardiol* 85: 195–197, 2002
39. Nielsen CB, Sorensen SS, Pedersen EB: Identical effects of indomethacin on renal function in healthy uninephrectomized subjects and in healthy control subjects. *Clin Sci* 86: 715–721, 1994
40. Moridaira K, Morrissey J, Fitzgerald M, Guo G, McCracken R, Tolley T, Klahr S: ACE inhibition increases expression of the ETB receptor in kidneys of mice with unilateral obstruction. *Am J Physiol* 284: F209–F217, 2003
41. Elijovich F, Laffer CL, Schiffrin EL, Gavras H, Amador E: Endothelin-aldosterone interaction and proteinuria in low-renin hypertension. *J Hypertens* 22: 573–582, 2004

42. Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, Fitzgerald GA: Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J Pharmacol Exp Ther* 289: 735–741, 1999
43. Taddei S, Vanhoutte PM: Endothelium-dependent contractions to endothelin in the rat aorta are mediated by thromboxane A2. *J Cardiovasc Pharmacol* 22[Suppl 8]: S328–S331, 1993
44. Taddei S, Vanhoutte PM: Role of endothelium in endothelin-evoked contractions in the rat aorta. *Hypertension* 21: 9–15, 1993
45. Hercule HC, Oyekan AO: Role of NO and cytochrome P-450-derived eicosanoids in ET-1-induced changes in intrarenal hemodynamics in rats. *Am J Physiol* 279: R2132–R2141, 2000
46. Trang AJ, Teoh H, Ward ME: Inhibition of prostanoid-mediated contraction to endothelin-1 after hypoxia in rat aorta. *Eur J Pharmacol* 423: 57–61, 2001
47. Newaz MA, Oyekan AO: Contribution of renal oxygenases to glycerol-induced acute renal failure in the rat. *J Cardiovasc Pharmacol* 39: 834–841, 2002
48. Oliver JJ, Rajapakse NW, Evans RG: Effects of indomethacin on responses of regional kidney perfusion to vasoactive agents in rabbits. *Clin Exp Pharmacol Physiol* 29: 873–879, 2002
49. Kotelevtsev K, Webb DJ: Endothelin as a natriuretic hormone: The case for a paracrine action mediated by nitric oxide. *Cardiovasc Res* 51: 481–488, 2001
50. Hoffman A, Abassi ZA, Brodsky S, Ramadan R, Winaver J: Mechanisms of big endothelin-1-induced diuresis and natriuresis: Role of ET(B) receptors. *Hypertension* 35: 732–739, 2000